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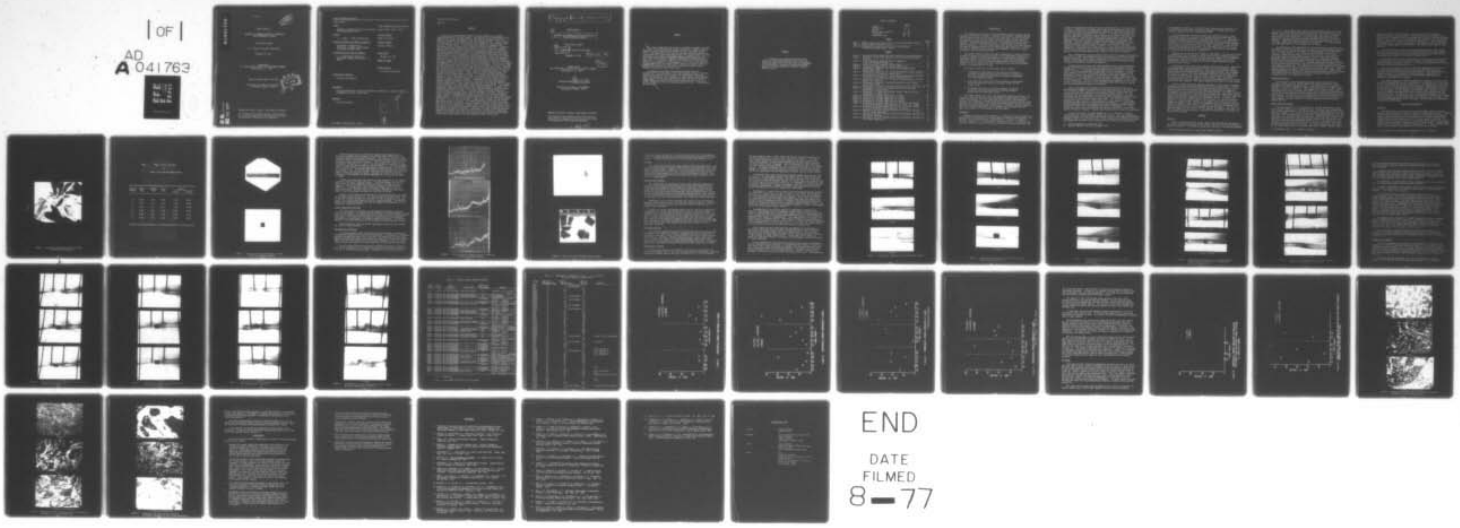

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Report Number 2

RESPONSE OF COMBINED ELECTRICAL STIMULATION AND BIODEGRADABLE CERAMIC

Second Annual Report

J. E. Lemons, Principal Investigator

December 15, 1976

Supported by
U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Washington, D.C. 20314

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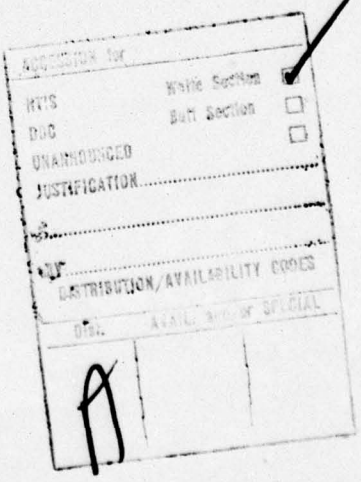

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ABSTRACT

Porous tricalcium phosphate ceramic has been proposed as a synthetic scaffold biomaterial for bone ingrowth. Applications for the replacement and/or augmentation of bone are suggested with the eventual biodegradation of the synthetic biomaterial important to long term biocompatibility. Initial studies include materials science evaluations of the biomaterial and an eighteen month 90 animal implant series. Sixty animals were studied for segmental bone replacements while 30 animals were studied for nonunion correction. Biomaterial analyses showed that comparisons of structure by x-ray diffraction produced differences in ralative peak intensities at selected 2θ angles but relatively consistent patterns sample to sample; an interconnected porosity average cross section exceeding 100 micrometers; the material could be fabricated to produce implant designs, and no difficulties were encountered in sterilizing or handling the material. The New Zealand White rabbit animal model provides an adequate model for initial studies on porous tricalcium phosphate ceramic for evaluation of tissue ingrowth, the role of this type of direct current electrical stimulation, biodegradation, tissue reaction, and nonunion replacements. The ability to remove the stabilization devices at 6 weeks for most of the rabbits is the earliest time we have experienced. Most "inert" porous implants require 12-16 weeks. Immediate post operative care was uneventful; however, some transcutaneous pin track infections were encountered after 3-6 weeks. These problems were severe for some of the long term nonunion animals. After removal of transcutaneous devices, the remainder of the animal care was routine. This porous tricalcium phosphate ceramic, in this animal model, can serve as a scaffold with bone proliferation through the large interconnecting pores. Transverse sections showed relatively complete ingrowth of bone at 12 weeks. Radiographs and gross observation at necropsy showed considerable variability in the rate of tricalcium phosphate implant biodegradation. Some animals retained most of the implant after 64 weeks of implantation while others showed almost complete biodegradation. The implants showed hard and/or soft conditions by sharp probe examination. In general, the radiographic appearance of the rabbit tibias showed a steady progression toward normal anatomy after 6-12 weeks. The direct current electrical stimulation resulted in more periosteal callus, and did not appear to greatly influence the tissue ingrowth and biodegradation rates for the porous tricalcium phosphate ceramic implants. Biomechanical strength comparisons from four point bending and determinations of the Work to Fracture of the lesion sites for implant and control conditions - at 3, 6, 12, and 64 weeks - showed similar ranges for the strength magnitudes at each time period. The average magnitudes of the Work to Fracture data increased with increasing time post surgery but showed a wide range within each group. Gross observation of implant sites at necropsy showed minimal tissue reaction while histological evaluations showed similar tissue characteristics for experimental and control conditions. Comparisons of porous tricalcium phosphate implants and implants plus direct current electrical stimulation at nonunions showed quite variable conditions and a very low probability for reestablishment of bony union after porous tricalcium phosphate ceramic implantation.

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SUMMARY

This report summarizes the results of 18 months of study on one form of a porous tricalcium phosphate ceramic biomaterial. Methods included materials science studies of the biomaterial and a 90 animal implant series. The biomaterial investigations included structural analyses by x-ray diffraction, quantitative measurements of the pore shapes and sizes, fabrication, and surgical environment handling conditions. The first year rabbit implant series compared 60 segmental lesion replacements in groups of 15: 5 control, 5 implant, and 5 implant plus direct current electrical stimulation (2-12 microampere) at 3, 6, 12, and 64 weeks post surgery. The second year implant series compared 30 nonunion animals, studying the potential for the porous tricalcium phosphate ceramic to develop a bony union at the lesion sites.

The segmental replacement investigations showed excellent biocompatibility for the porous tricalcium phosphate ceramic, relatively complete bone ingrowth after 12 weeks, and considerable promise for future applications of this biomaterial in maxillofacial and orthopaedic surgery. The nonunion investigations show that additional research is needed in order to optimize the surgical methods for the use of this synthetic biomaterial for this particular type of lesion. Investigations are continuing.

FOREWORD

In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences - National Research Council.

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INTRODUCTION

The treatment of maxillofacial and orthopaedic skeletal injuries has been changing over the years; however, in general, the surgeons' treatment depends upon the reduction and stabilization of the injury until the site has healed. The treatment of bone loss and severe trauma most often includes surgical procedures and autogeneous bone grafting. The autogeneous bone graft usually requires a second and independent surgery in conjunction with the primary surgical procedure. Long term immobilization and treatment are often required. The research program presented in this report has major objectives to shorten the time of treatment of maxillofacial and orthopaedic injuries, to reduce deformities, and to decrease morbidity.

The present research project evaluates a combination of known techniques in order to improve the prospects for the use of biodegradable materials as a substitute biomaterial for the development of new bone growth at surgically introduced lesions. Although each technique in itself has considerable importance, the combined use of porous biodegradable tricalcium phosphate ceramic and direct current electrical stimulation might provide advantageous effects to eliminate defects in bone.

The technical objectives of this project are as follows:

1. To develop an animal model for the study of the combined techniques of applying direct current electrical stimulation to tissue ingrowth and stabilization of porous biodegradable tricalcium phosphate ceramic.

2. To evaluate the kinetics and tissue response to biodegradable tricalcium phosphate ceramics at surgical lesions in the animal system.

3. To evaluate the kinetics and tissue response of combined electrical stimulation and porous tricalcium phosphate ceramic with the same animal system.

This report combines the first[1] and second year research activities. The first year emphasized porous tricalcium phosphate ceramic segmental implants placed into surgical sites that would heal with or without the biomaterial implant. The influence of externally applied electrical stimulation was one of the conditions investigated. The second year program emphasizes the long term implants (15 months) and possible applications to surgically produced nonunions. Many of the nonunion experiments are in-progress and the results on these particular animals will be included in the next report.

Background

Research on porous synthetic materials as scaffold for tissue ingrowth has demonstrated the applicability for these types of biomaterial applications. The materials used as scaffolds have included metals,[2,3] alloys,[4,5] ceramics,[6] carbons,[6] polymers,[7,8] and composites of these materials.[9,10] In general, the more inert materials have been used in an attempt to minimize tissue reactions

related to biodegradation products from the implant materials. The significant increase in surface area associated with highly porous biomaterials may result in increased quantitites of biodegradation products in the bioenvironment and a potential for latent biocompatibility problems. To minimize the possibility of adverse biodegradation products, many of the materials used for scaffolds have been the more inert alumina and titania ceramics and pyrolytic and vitreous forms of carbon. These materials, although extremely inert, show very little deformability without fracture in comparison to metallic and polymeric biomaterials. With the presently available "inert" scaffold materials and fabrication technologies, possibilities for fracture or failure of these type scaffolds at relatively low stresses do exist. This is quite critical when the biomaterial is considered for use with bone or in other high stress applications. If the scaffold materials were to fail, formerly viable tissue within the biomaterial structure might become necrotic and act as a nidus for infection.

Thus, although there are applications for the inert porous scaffold materials, there also is a need for biomaterials that could serve as a scaffold and subsequently biodegrade. These types of biomaterials would provide a site for the initial tissue ingrowth and stabilization, with the scaffold eventually being resorbed and replaced by normal body components.

Investigations have shown that selected compositions of calcium-phosphate ceramics provide characteristics for consideration as biodegradable scaffold biomaterials.[11,12] Tricalcium phosphate showed sufficient promise such that routine fabrication and production techniques have been developed. A company* and a research institute** have prepared porous tricalcium phosphate ceramic biomaterials that are now available to the medical and dental community. The availability of this type material, where the routine quality control would be maintained, is quite important to the consideration of widespread clinical usage of this biomaterial. Since the tricalcium phosphate biodegrades, a first consideration is the potential for toxicity problems related to the material *per se* and the biodegradation products of the material.

Animal investigations conducted at the United States Army Institute of Dental Research (USAIDR) and supported by USAIDR have shown that the constituents of tricalcium phosphate ceramic and tricalcium phosphate ceramic coated with various materials were compatible with laboratory animal systems.[13-18] The substances from the biodegradable ceramic investigated during 1971-1973 were eliminated from the physiological environments within reasonable lengths of time and no local or organ related effects were noted. This research has been continued through a series of animal models and in most cases the tricalcium phosphate has been shown to be physiologically acceptable.[19-21] Investigations on the biodegradable calcium phosphates from other laboratories have provided additional insight into the biocompatibility of this biomaterial.[22-28] These studies were not directly supported by the USAIDR and have been a natural outgrowth of research interests. Research and limited clinical trials are continuing.

In most applications of porous biomaterials, it is desirable to obtain stabilization by tissue ingrowth as rapidly as possible. Also, as a resorbable scaffold degrades, the bone should proliferate to replace the biodegrading scaffold. Methods to enhance or partially control tissue ingrowth, ossification and continued development may therefore play an important role in improving the applicability

* Miter Incorporated, Worthington, Ohio.
** Battelle Memorial Institute, Columbus, Ohio.

of biodegradable scaffolds. One possibility of improving the conditions for the application of the porous tricalcium phosphate is to combine it with externally applied electrical stimulation of bone.

Investigations over the past ten years have shown that direct, pulsed, alternating, and various combinations of applied electrical stimulation can lead to considerable enhancement in the formation of bone.[29-31] Experimental techniques for enhanced bone callus formation have been described and several laboratories report 30-50% increases in the amount of bone and 30-50% decreases in the time to clinical union at fracture sites. Additionally, some nonunion sites have been successfully treated. Thus, the amount and the site of bone formation or bone healing can be, to some extent, controlled by the presence of externally applied electrical stimulation.

Research at the University of Alabama in Birmingham,[32] over the past 4 years, has demonstrated that the amount of callus within a large transverse surgical defect in rabbit tibias could be increased by direct current (2-12 microampere) stimulation. Additionally, these studies showed the feasibility of using biomechanical testing to evaluate the relative strength characteristics of these particular lesions.

An initial one year study of the combined effects of electrical stimulation and biodegradable tricalcium phosphate ceramic was initiated using a rabbit animal model and established conditions. The established conditions included the availability of biodegradable tricalcium phosphate ceramic, the biocompatibility of this material as a bone replacement substance, and the known effect of forming considerable bone callus by direct current electrical stimulation. The results of this initial study were quite favorable and a second year program associated with the potential of tricalcium phosphate for the correction of nonunions was conducted. This report summarizes the results of these studies.

With the extensive research programs conducted on the tricalcium phosphate bioceramic, one might speculate about possible clinical applications. Clinical applications of materials of this type might include filling in various cystic lesions of bone such as unicameral and aneurysmal bone cysts and segmental replacements for such lesions as giant cell tumors of bone. The latter situation is particularly difficult in that with present methods the involved bone must be replaced with homograft requiring prolonged immobilization. Often the inadequate support and stability provided by this replacement results in collapse and deformity of the graft and/or adjacent joint surfaces. An ability to provide support, to maintain normal anatomy, or to support joint surfaces while bone regenerates would provide a significant improvement over any presently available method of treatment. Often there is a need for an additional autogeneous bone graft during maxillofacial and orthopaedic surgical procedures. The tricalcium phosphate might serve the role as a filler material for needs of this type. If this biomaterial proves successful, the potential applications in dentistry and medicine are extensive.

METHODS

Materials

Porous tricalcium phosphate ceramic samples were obtained from two sources. The samples for the major portion of the animal study were obtained from Battelle Memorial Institute.* The Battelle samples were supplied in rod form approximately

* Ceramic Materials Section: Larry McCoy, Research Ceramist.

1.2 x 10 cm. These samples were obtained at three different times within the program. The initial group was received during the first quarter of the first year, a second group near the end of the first year, and a final group near the mid-point of the second year. All samples were processed in the same laboratory, from the same starting powders and under the control of the same ceramist. Independent samples of porous tricalcium phosphate ceramic for the first year studies were obtained from Miter Corporation.* The Miter samples were rods approximately 2.0 cm diameter by 3 cm length.

The laboratories of Battelle Memorial Institute and Miter Corporation were responsible for the quality control of the porous tricalcium phosphate materials. As a cross check on the quality control and intercomparability of these materials, a limited number of x-ray diffraction and microscopic studies were conducted. Powder pattern peak intensity versus 2θ angle x-ray diffraction scans were made on selected samples using a Phillips x-ray diffractometer. Microscopy studies of fracture surfaces were done on a Cambridge Stereoscan Mark II Scanning Electron Microscope (SEM) after vacuum coating the fracture surfaces with a gold-palladium alloy. Standard comparison photographs were made at 56x and 1100x magnifications. Samples were also mounted for cross section metallographic examination. Polished plane sections were examined using reflected light illumination on a Bausch and Lomb or a Leitz optical microscope. Standard photomicrographs were taken to show the sample microstructures. Quantitative stereology methods for average line length occupied (volume fraction) and average line length within the large pores were used to develop histograms of the frequency of pore size versus pore size data.

Implant Fabrication

The rod shaped tricalcium phosphate samples were sectioned into one centimeter lengths and bone implant samples fabricated using dental cutting instruments and diamond sectioning discs. Each sample was cut to produce an implant of a specific "anatomical" shape, approximately 8 mm in length, cleaned, weighted, and dry heat sterilized. The implants for the bone surgical lesion implantation (first year) were fabricated with small projections along each end so that the implant would be partially stabilized in correct anatomical position after surgery. The implants for the nonunion sites did not have projections, in that there was no site for projection placement within the bone structure. All chips, powders, and extra pieces of tricalcium phosphate were maintained as implant material.

Animal Model and Surgery

The animal model for this investigation was the New Zealand White rabbit. The as-received animals were 4-6 months of age. The animals were anesthesized using sodium pentobarbital via the ear veins and the animal prepared for sterile surgical technique. The surgical site was the tibio-fibular junction of the left rear limb. This site was centralized with four threaded Steinmann pins with the two central pins providing sites for electrical stimulation when required. The outermost pins were driven completely through the bone while the central pins were driven from one side only engaging both cortices. The central pins were insulated to the bone surface and the four pins were held together with acrylic splints placed externally to the animal. The surgical defect was just distal to the tibio-fibular junction, the defect was equal distances from each central

* Worthington, Ohio: T.D. Driskell, President.

pin and was sized to fit the implant. During the first year program, a segment of bone was removed surgically, the periosteum was not removed, and the surgically introduced lesion was replaced with a porous tricalcium phosphate implant. The variability in bone anatomy and the shape of the implant required that the weight of the implant be used for standardization. Each implant was fabricated to be the same size and shape with the shape replicating the anatomy of the bone segment removed from the tibia. The implants were placed using a tight fit.

The initial series of porous tricalcium phosphate implants were placed within the surgical defect without infiltrating with saline or other solutions. The second series of porous tricalcium phosphate implants (40 animal series) were placed after vacuum infiltration with normal saline.*

After placing the implant, the subcutaneous tissues were closed using 4-0 chromic catgut and the skin closed with 3-0 Dexon. An example of the animal leg during surgery is shown in Figure 1. The rabbits were allowed full activity post operatively and food and water ad lib.

When utilized, the direct current electrical stimulation was applied to the two central stabilization pins using two size AA dry cell batteries connected in series with a 500 kohm variable resistor. The battery pack and current magnitude, 4-6 microamperes, were checked on a regular basis for integrity. Minor adjustments of the variable resistor were required to compensate for changes in the system.

The animals prepared for nonunion studies were treated in a similar manner for surgical preparation and bone stabilization. In contrast to the first year studies, the bone lesion size was increased to 12 millimeters and the periosteum at the surgical site was removed by sharp dissection. Subcutaneous closure of the tissues for the nonunion group was accomplished using 4-0 Dexon rather than 4-0 chromic catgut. If the surgical site developed into a nonunion, a second surgical procedure was conducted to place a porous tricalcium phosphate into the nonunion site. The ends of the bone were resected to produce a correctly sized implant site. In some cases, where a nonunion was not formed and a bridge of bone connected the distal and proximal bone segments, chips or powders of the tricalcium phosphate were surgically implanted. All sections were photographed and submitted to histology.

RESULTS AND DISCUSSION

Materials

The number, source, and type of tricalcium phosphate samples provided by Battelle Laboratories for the nonunion Studies are summarized in Table I. Examples of the as-received samples are shown in Figure 2. The samples fabricated to produce implants were found to be relatively uniform in their microstructural characteristics and the shapes and ability to carve caused no problems. All samples showed a slight skin effect, or a relatively dense layer on the surface. Therefore the surfaces were removed during implant fabrication. Selected samples showed internal structural variability (reported by Mr. L. McCoy, Battelle) and large implant pieces of these samples were not used in our studies.

* This procedure was adopted at the suggestion of Dr. L. Getter.

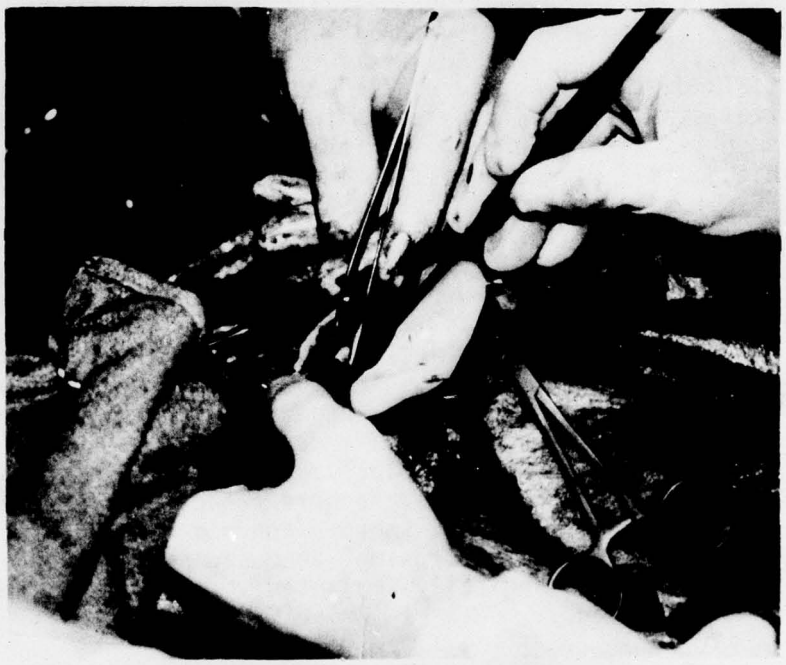
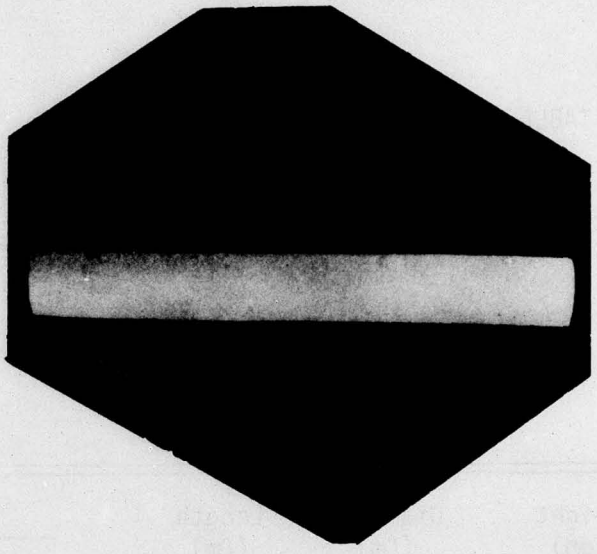


FIGURE 1. New Zealand White Rabbit Left Rear Limb During Surgical Procedure.

TABLE I. Number, Source, and Type of Porous Tricalcium Phosphate Samples

Specimen Number	Weight (gm)	Diameter (Cm)	Length (Cm)	Density	
				gm/Cm^3	Percent
2	22.82	1.32	10.95	1.516	48.27
10	9.02	1.00	7.33	1.564	49.80
11	16.41	1.11	10.71	1.615	51.42
12	13.64	1.07	9.83	1.546	49.25
14	13.09	1.08	9.58	1.503	47.87
15	13.04	1.06	9.45	1.552	49.42



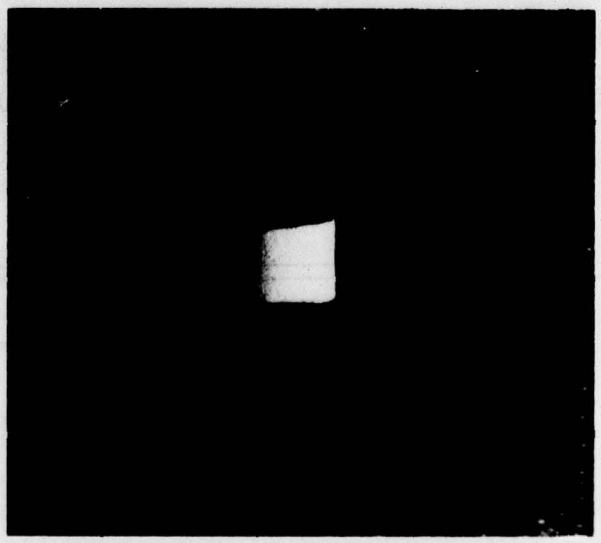


FIGURE 2. Examples of As-received Samples of Porous Tricalcium Phosphate Ceramics.

Microstructural and x-ray diffraction studies were conducted on the tricalcium phosphate ceramic samples as a quality control cross-check. The microscopy studies were reported previously.[1] The relative x-ray diffraction intensity versus 2θ angle analyses for the Battelle and Miter porous tricalcium phosphate ceramics are shown in Figure 3. The relative peak intensities and 2θ locations for materials "1" and "3" are similar while in contrast the peak intensities are much less defined for material number "2." One would speculate that the volume fraction of organized crystalline constituent in sample number "2" was much lower. The nominal chemistries on these materials were listed as being similar. One can only conclude, therefore, that processing variables can greatly influence the material per se. How much this type of variability influences the biocompatibility and biodegradability of tricalcium phosphate is unknown.

Implants were fabricated from the most similar materials and the implant shapes were made to suit the application. The implant shapes for the first year studies of tibial segmental lesions are shown in the upper portion of Figure 4. Sections of rabbit tibia removed at surgery are included for comparison. The implants were made to anatomical shape where a tight fit could be obtained. The small tips on the implants extended into the marrow space of each bone segment and helped to stabilize the implant.

Examples of the shapes of the implants for the nonunion studies are shown in the lower portion of Figure 4. The rods were used in nonunion sites. Tips on the ends of these implants were not useful in that the surgery on the bone ends did not expose marrow spaces during the implant procedure. The chips and powders of tricalcium phosphate were used for augmentation procedures in the second year (nonunion) studies.

Animal Surgery and Follow-up

The rabbit surgical procedures and post operative recovery period was relatively uneventful. The anesthesia was sufficient to provide adequate surgical time and the animals moved around within their cages shortly after completion of the surgery. The rabbits were followed on a daily basis by the Biomaterials Laboratory and the Department of Comparative Medicine personnel. The specific details of the animal follow-up will be included with each subsequent section.

Anterior-posterior (AP) and oblique radiographs provided the best method for routine animal evaluation

Post Operative Follow-up

All animal procedures were approved by and checked through the Department of Comparative Medicine with the staff veterinarians. The biomaterials laboratory personnel followed the animals on a daily basis for observation and evaluation. Weekly radiographs were made on all animals for times up to 6 weeks post surgery. Some series were then discontinued for 3-4 weeks intervals, if the pin stabilization systems were removed or if the animals were in the nonunion study group.

The first year animals were separated into groups of fifteen (5 controls, 5 tricalcium phosphate, and 5 tricalcium phosphate plus electrical stimulation) at 3, 6, 12, and 64 weeks post surgery. The animals in the nonunion series

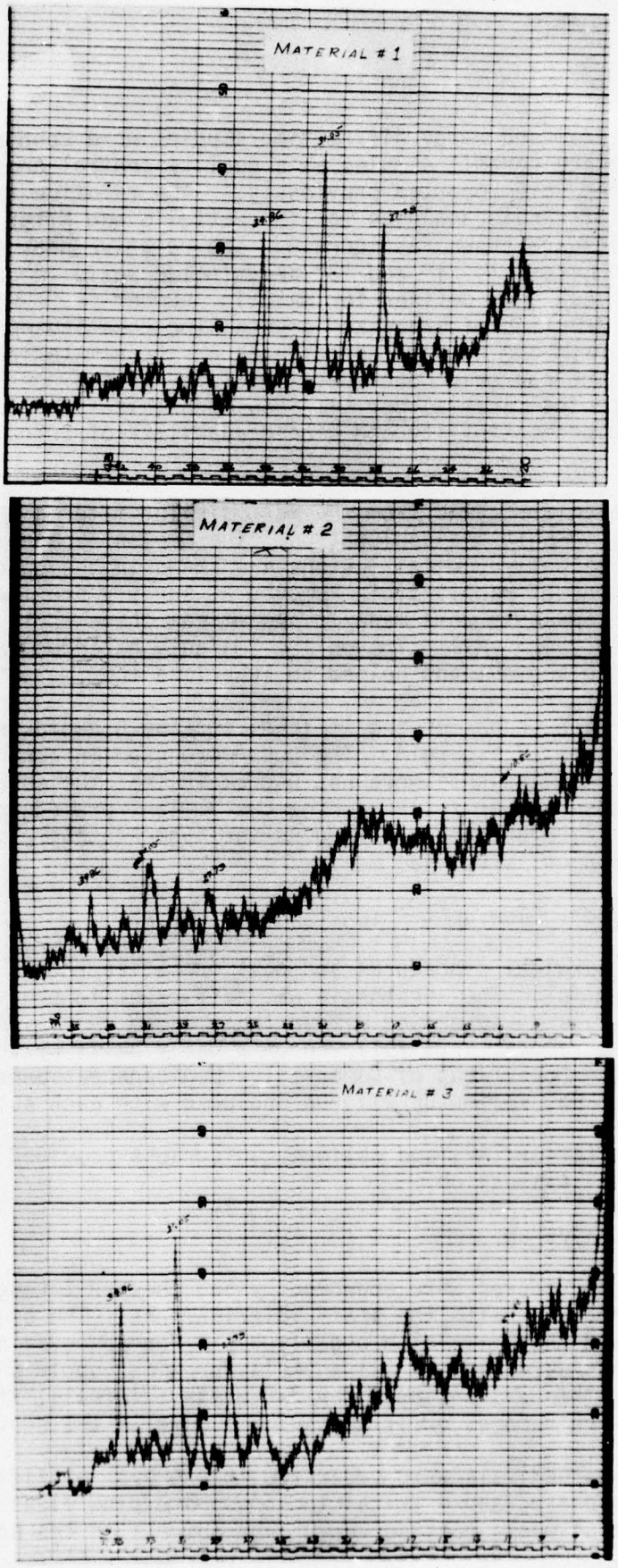
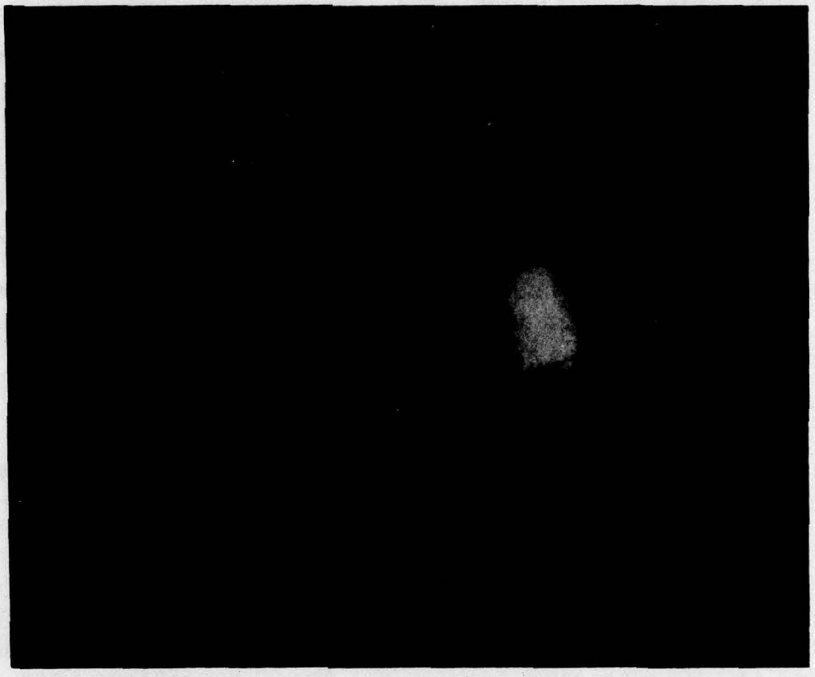


FIGURE 3. X-ray Diffraction Intensity Versus 2θ Angle for Tricalcium Phosphate Ceramics.



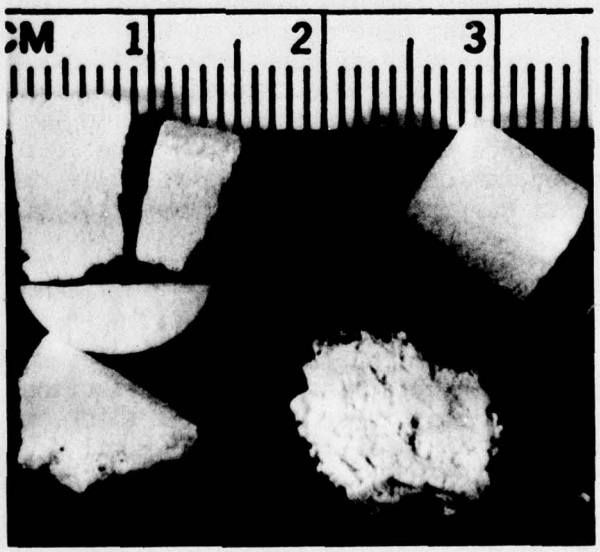


FIGURE 4. Porous Tricalcium Phosphate Implant Shapes.

(second year) were followed until a union or nonunion of the tibia developed. Surgery was conducted after the site stabilized following previously described methods. Follow-up then resumed on an active one-per-week radiograph basis.

Necropsy

The animals were euthanized by drug overdose after gross observation. The tibia and other tissues of interest were removed by sharp dissection and histological specimens taken. Examples of tibias and associated tissues were photographed using 35 mm color slide film. The contralateral tibia was removed if the specimen showed apparent bone like (rigid) biomechanical strength. Each removed tibia was photographed using a Polaroid MP-4 camera system and 55 P/N film for record purposes.

Biomechanical Strength

The experimental and contralateral tibias were tested for biomechanical strength using an Instron Testing machine and a four point bending fixture. A 200 pound scale compression cell range and a head rate of 0.05 inches per minute were utilized for all testing. The four point bending test fixture utilized roller contact points and dimensions that developed fractures of the lesion sites near the centers of the previous surgical lesions. The influence of the stabilization pin sites and the variable anatomy of the rabbit tibias are minimized with this testing technique. Moist conditions were maintained.

Immediately after fracture, the bones were fixed in 10 per cent neutral buffered formalin and the area of the fracture surface determined. The force, deformation, and fracture surface area data was reduced to develop Work to Fracture (W_f) measurements.

The Work to Fracture measurements depend upon the load versus deformation relationship for the four point bending test as well as the bone fracture interface area. The determination of bone interface area for the callus type lesions is quite difficult and introduces considerable variability into these data. The bone sample evaluations for the longer time implantations reduce this error due to the change from woven (callus) type bone to more compact type bone. Because of the anatomical variabilities found in rabbit tibias, the Work to Fracture measurements have proven to be the most satisfactory of the techniques evaluated.

Histology Specimens

The fixed bone lesions from the first year studies were sectioned proximal and distal to the central pin locations to produce sections for reduction to histological specimens. Slices, three millimeter thick, were removed from the lesion site at a position 1 centimer from the proximal central stabilization pin. The bone, soft tissue, and tricalcium phosphate ceramic regions were maintained by sectioning with a diamond disc and jewler's hacksaw. The nonunion samples were sectioned at positions to provide the most representative sample.

Radiographic Follow-up

The first year studies of the control (with lesion but no implant), the tricalcium phosphate implant, and the tricalcium phosphate implant plus electrical stimulation compared implant conditions in animals at 3, 6, 12, and 64 weeks.

The post surgery (3 day), 6 week (after removing the stabilization pins), and 64 week radiographs for a control rabbit are shown in Figure 5. The 3 day post surgical radiograph shows the approximately 8 mm length lesion and the stabilization device. The periosteum remained and the bone rapidly healed as is noted in the 6-7 week radiograph. The 6-7 radiograph was taken just after the stabilization pins were removed. This situation was average for the control animals. The bottom radiograph, Figure 5, was taken at 64 weeks and the bone anatomy has returned to a relatively normal condition. This will be discussed further in association with the biomechanical strength testing results section.

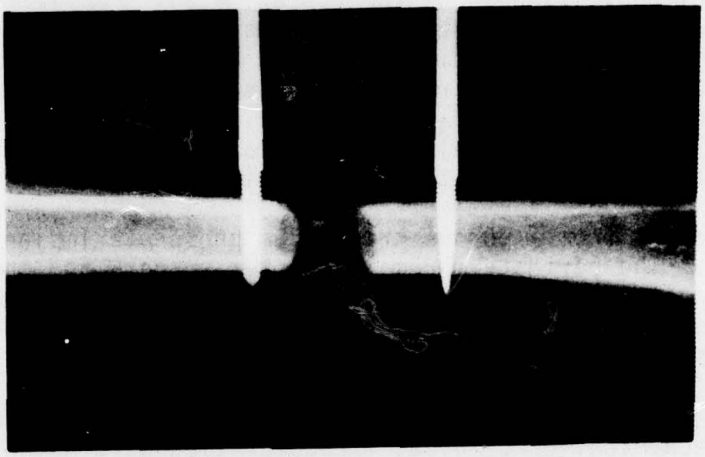
Radiographs from a porous tricalcium phosphate implant condition are shown in Figure 6. The periosteum remained after surgery and the stabilization pins were removed at approximately 6 weeks. The role of the small tips or the ends of the porous tricalcium phosphate implants are clearly seen as an extension into the marrow space on each side of the lesion. The radiograph at 64 weeks was made at necropsy after the soft tissues were removed. Note that the implant had not disappeared (biodegraded). However, the rabbit limb was completely functional and no biomechanical or clinical irregularities were noted.

Radiographs of a rabbit with a porous tricalcium phosphate implant combined with electrical stimulation are shown in Figure 7. The view of the implant in the 3 day post surgery radiograph does not show the tips on the implant because of a slight anterior-posterior angle of the implant. At six weeks the implant is surrounded by bone and at 64 weeks the implant area shows what appears to be residual tricalcium phosphate ceramic. In general, the radiographs showed more periosteal callus associated with the electrically stimulated implant sites. The electrical stimulation, however, did not appear to greatly influence the biodegradation rate of the porous ceramic implants.

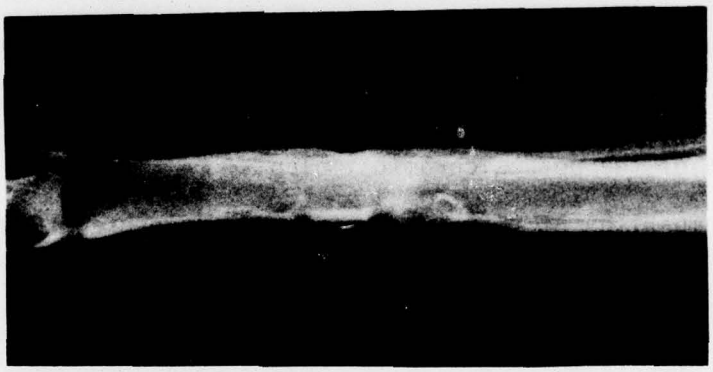
For comparison of the relative amounts of degradation, radiographs of electrically stimulated tricalcium phosphate ceramic implant sites are shown in Figure 8. Rabbit R154E shows a significant quantity of tricalcium phosphate loss at the implant site while in contrast Rabbit R150E shows only a slight change in appearance with respect to biodegradation. This same trend was also noted in the nonelectrically stimulated rabbits. Radiographs of examples for two nonelectrically stimulated implant sites are shown in Figure 9. Rabbit R145 shows relatively little biodegradation at 64 weeks post surgery. In all cases the rabbits showed full functional abilities and no observable clinical irregularities.

The follow-up for this animal group was uneventful with the exception of death before the euthanization schedule and some problems in animal inventory. The animals are changed between cages on a regular basis to clean the cages. These transfers for large number of animals, by the cleaning personnel, resulted in some problems in animal identification. In one case, over a weekend, an animal was found dead, placed in the morgue, and incinerated without notification of project personnel.

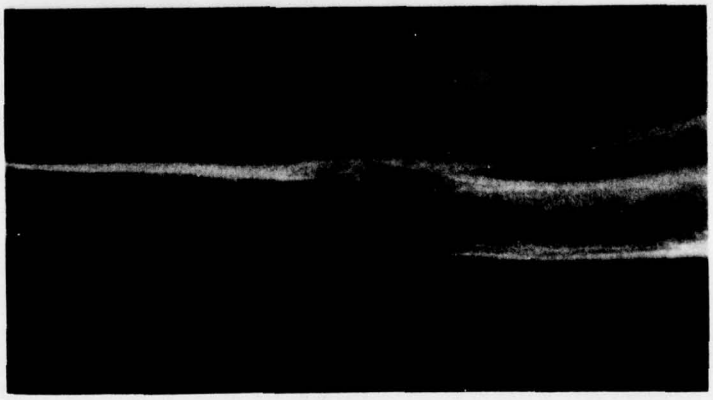
Chronic problems with the long term follow-up on the nonunion investigation rabbits created difficult situations. The transcutaneous threaded pin stabilization devices were a continual source of problems. The transcutaneous pin sites were a source for chronic irritation and several of the animals were eventually euthanized because of pin track related infections. Another problem was the breakage of the stabilization pins after extended periods of use. These problems



3 days

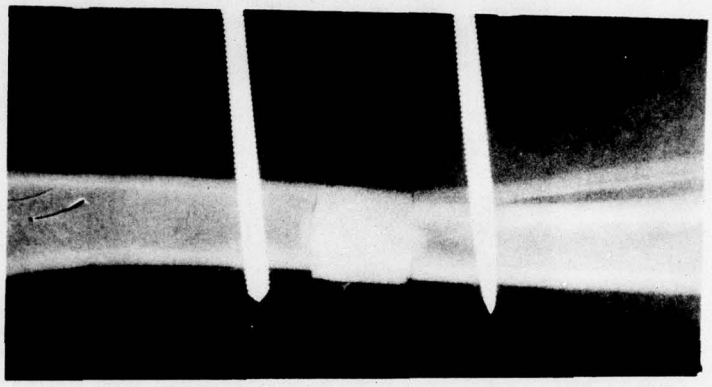


6 weeks

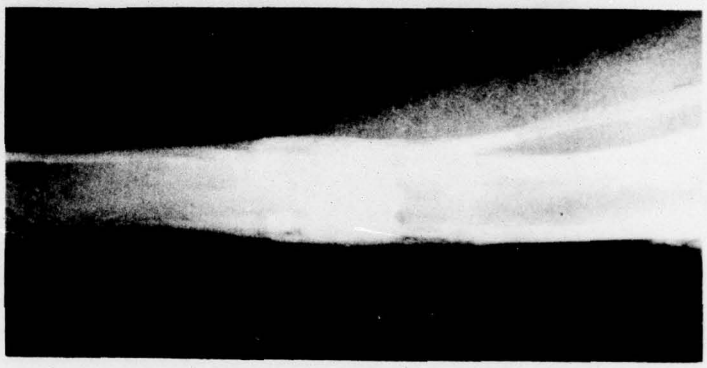


64 weeks

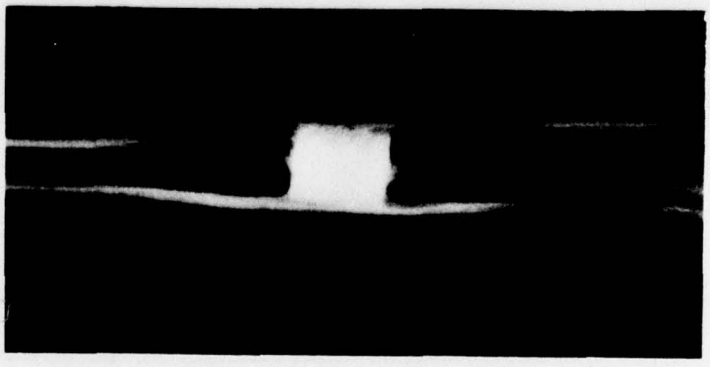
FIGURE 5. Radiographic Comparisons for Control Rabbit R118C.



3 days

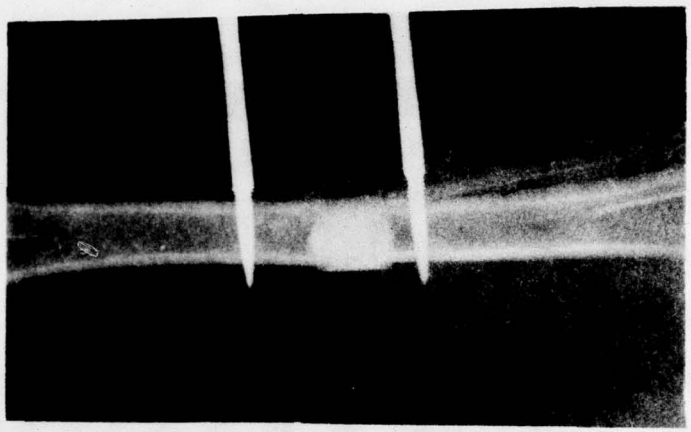


6 weeks



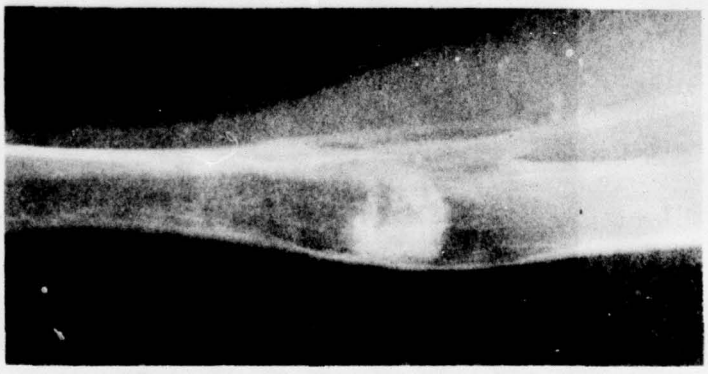
64 weeks

FIGURE 6. Radiographic Comparisons for Tricalcium Phosphate Ceramic Implant Rabbit R144.



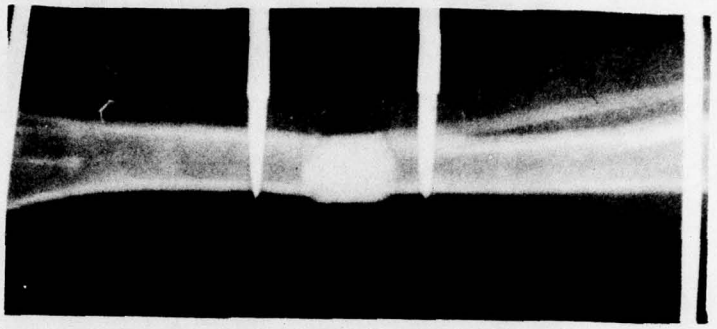
3 days

6 weeks

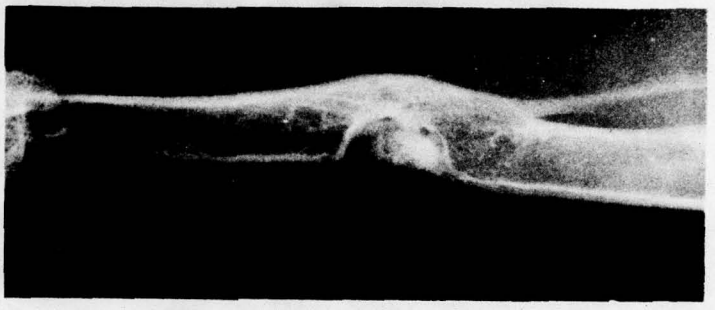


64 weeks

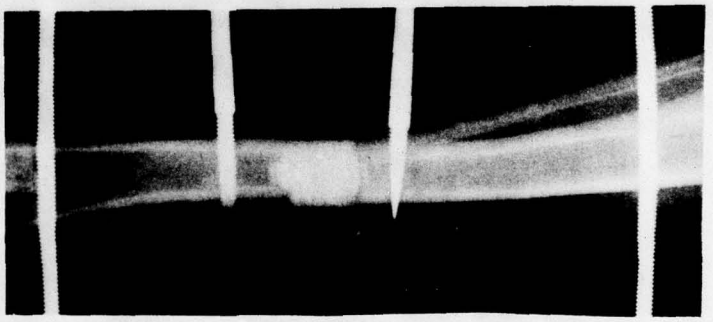
FIGURE 7: Radiographic Comparisons for Tricalcium Phosphate Ceramic Combined with Electrical Stimulation, Rabbit R152E



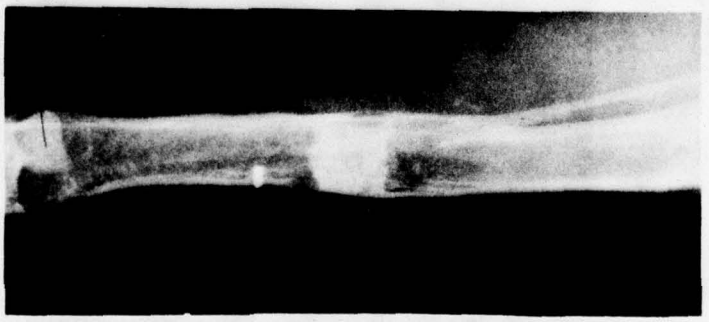
R154E - 3 days



R154E - 64 weeks

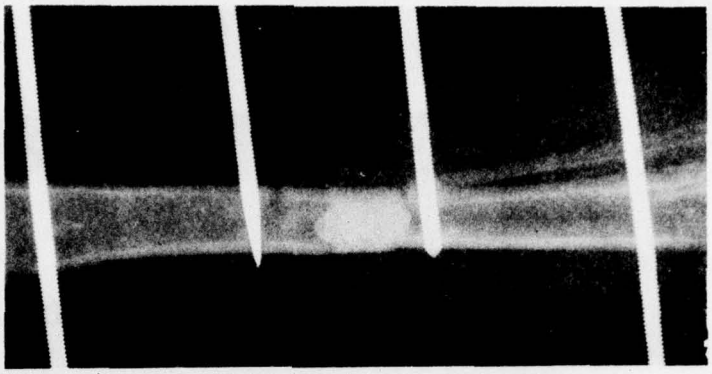


R150E - 3 days

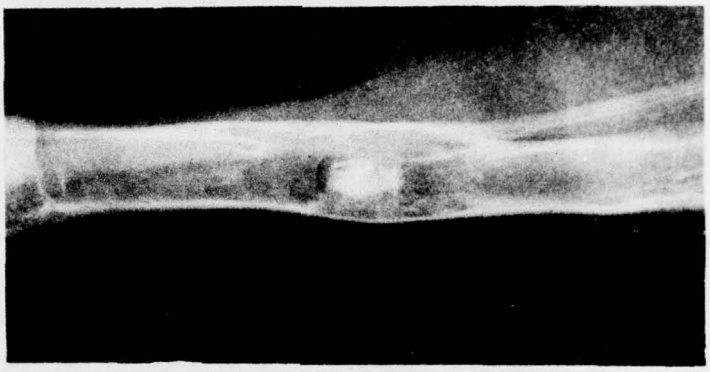


R150E - 64 weeks

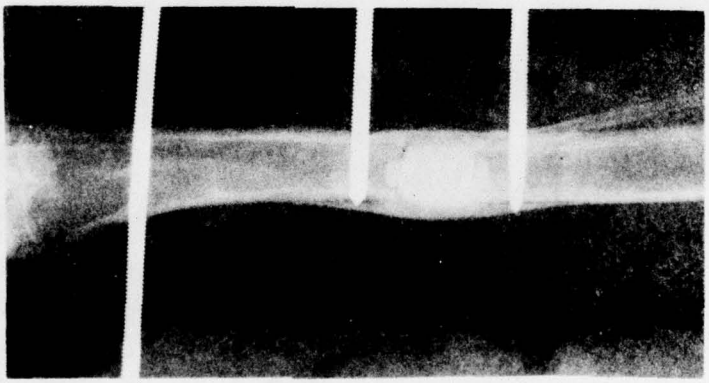
FIGURE 8: Radiographic Comparisons for Tricalcium Phosphate Ceramic Combined with Electrical Stimulation, Rabbits R154E and R150E



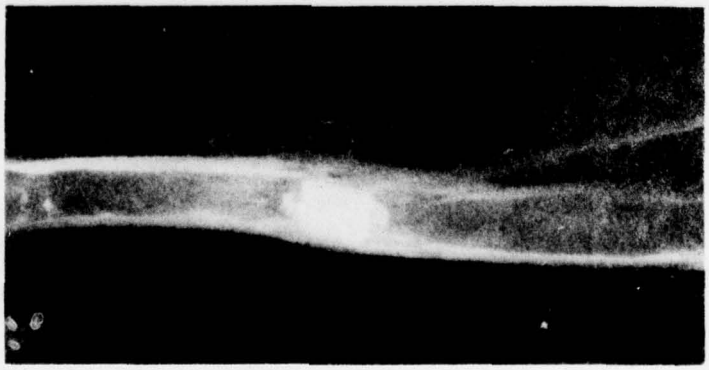
R145 - 3 days



R145 - 50 Weeks



R139 - 3 days



R139 - 64 weeks

FIGURE 9: Radiographic Comparisons for Tricalcium Phosphate Ceramic Implant Rabbits R145 and R139.

did not prevent the objectives and specific aims of the project from being satisfied; however, they did require considerable time input from a routine care standpoint.

Radiographs from a nonunion rabbit receiving a porous tricalcium phosphate ceramic implant at approximately 6 months post surgery are shown in Figure 10. The radiograph after 4 months of porous tricalcium phosphate implantation shows bone associated with the implant site. General radiographic evaluation of this site caused the retention of the stabilization system.

For comparison, a nonunion series with an appearance of less bone associated with the tricalcium phosphate implant is shown in Figure 11.

In general, radiographs of the nonunion implant animals showed an incomplete healing of the nonunion sites associated with the porous tricalcium phosphate implants. These studies are continuing.

A number of the animals developed a narrow bridge of bone across the proposed nonunion site. Within this study period, the bridge of bone did not mature enough to develop sufficient biomechanical strength to attempt removal of the stabilization devices. To utilize these animals, large chips and/or powders of the porous tricalcium phosphate implants were implanted as a bone augmentation study series. Radiographs of one example of chips implanted into a rabbit lesion site are shown in Figure 12. The bridged "nonunion" site is shown in the upper radiograph, implants at 2 months in the middle, and the implant site at 5 months post implantation in the lower radiograph. The tricalcium phosphate chips appear to be slowly biodegrading. However, the bone structure at 5 months post implantation is still insufficient to permit the removal of the stabilization device.

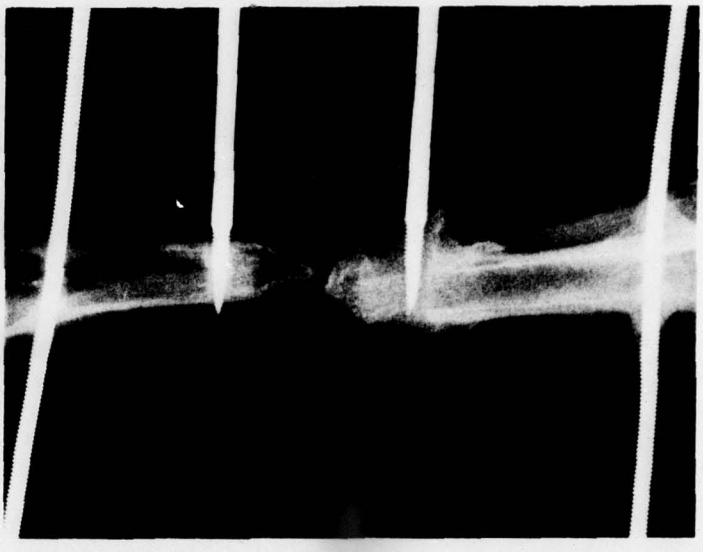
Radiographs showing an example of a powder (granular) tricalcium phosphate implant augmentation are shown in Figure 13. The bridged and implant site is shown at 3 months and the final (lower) radiograph is shown at 4 months post implantation. Most of the powder (granular) material appears biodegraded. In this case, the bone showed sufficient biomechanical strength to remove the stabilization system. This experiment is continuing.

Many of the various nonunion specimens are now being evaluated using histological sections to determine the tissue conditions. The animal number, experiment type, and disposition for the second year animal series are summarized in Table II.

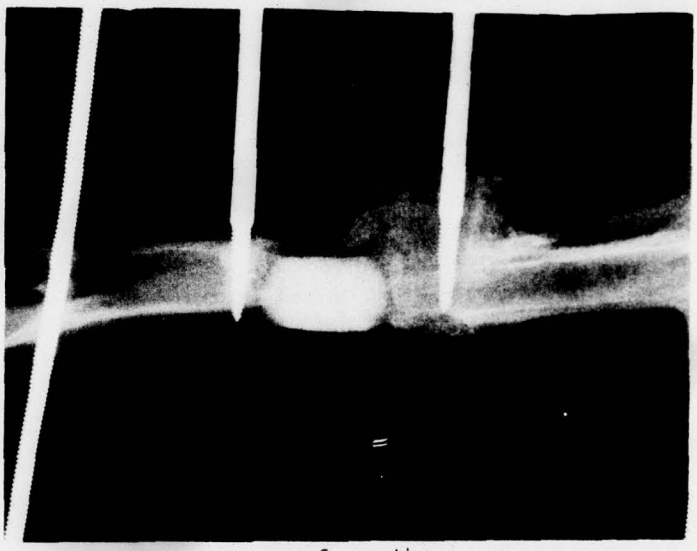
Biomechanical Strength

The rabbit experimental conditions and the biomechanical strength data are summarized for the first year investigations in Table III. These data are shown in graphical form in Figures 14-17. The three week post surgery period shows a comparison of Work to Fracture for the control, the tricalcium phosphate implant, and the implant with electrical stimulation conditions. All of these lesion sites were low in strength and none of the stabilization systems could be removed at this time.

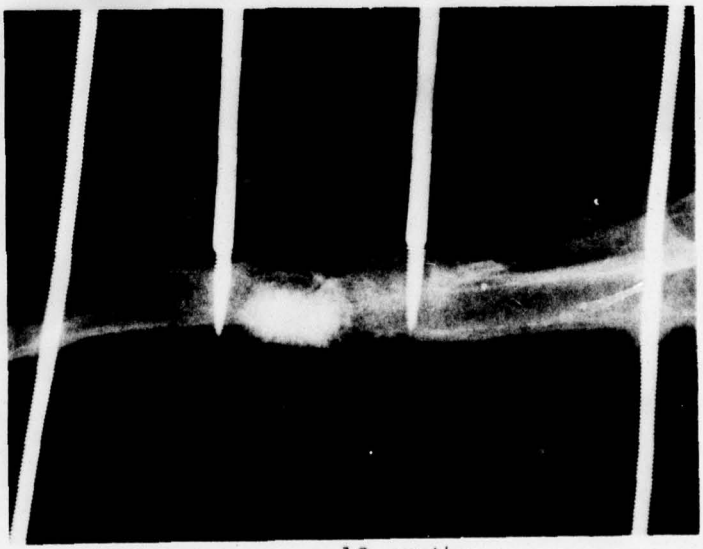
The Work to Fracture comparisons at 6, 12, and 64 weeks post surgery (Figures 15, 16, and 17) show similar magnitude strengths for all conditions



6 months

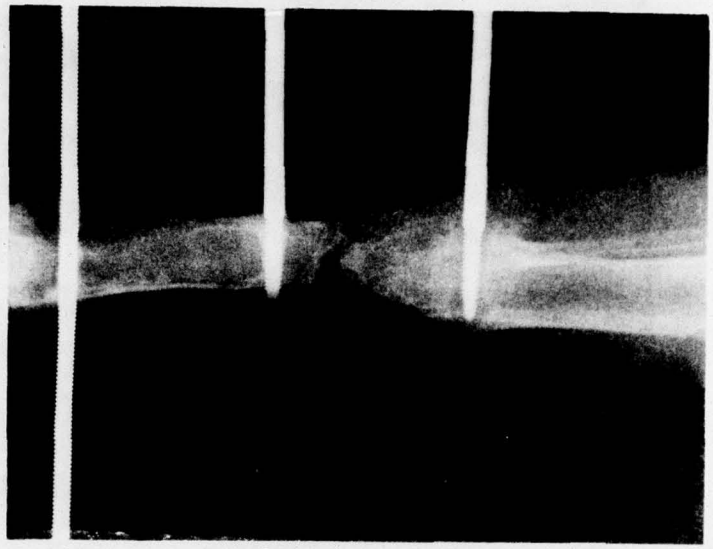


6 months

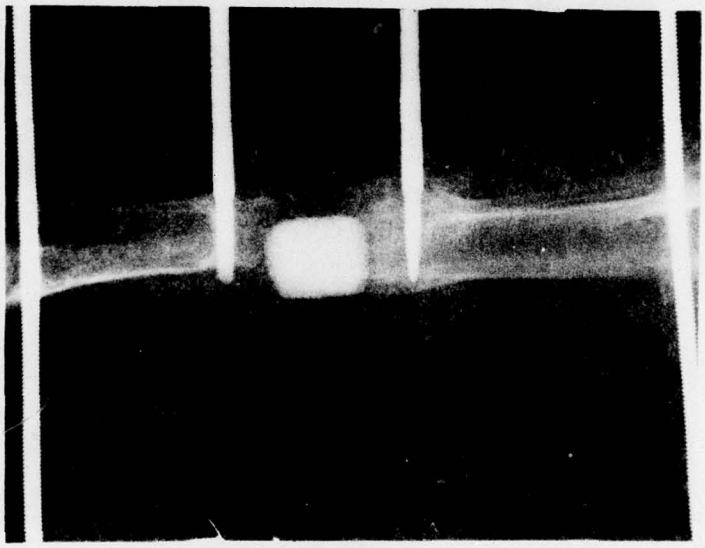


10 months

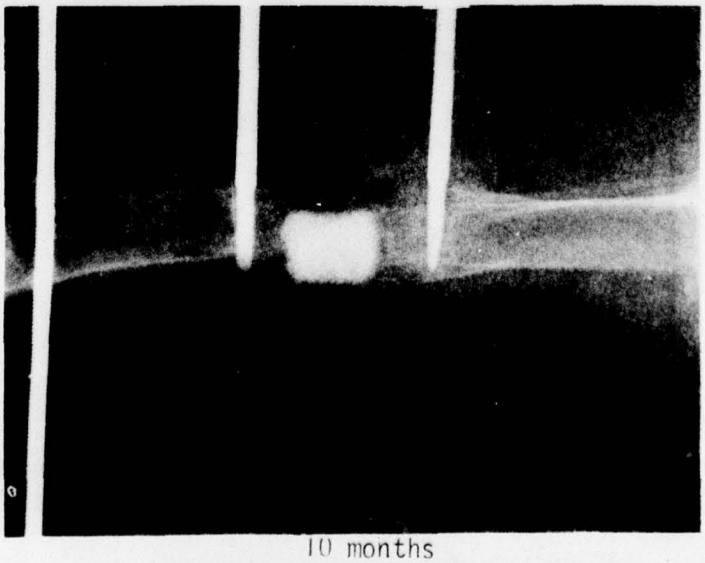
FIGURE 10: Radiographs of Nonunion Tricalcium Phosphate Ceramic Implant, Rabbit Number R208.



6 months

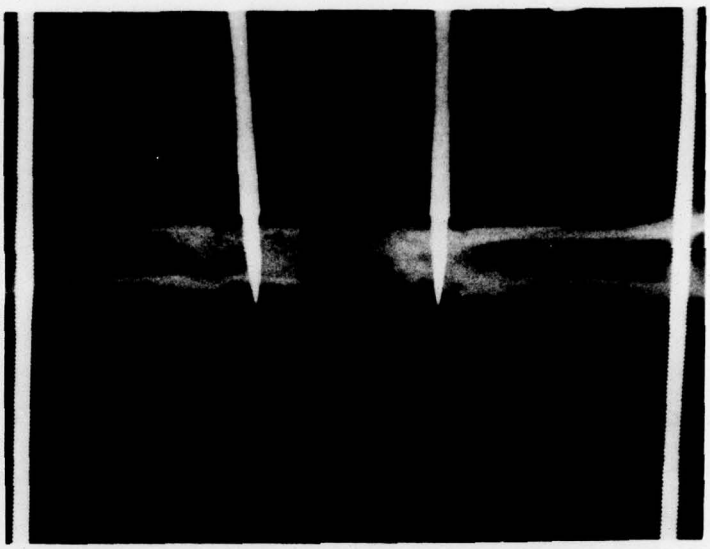


6 months

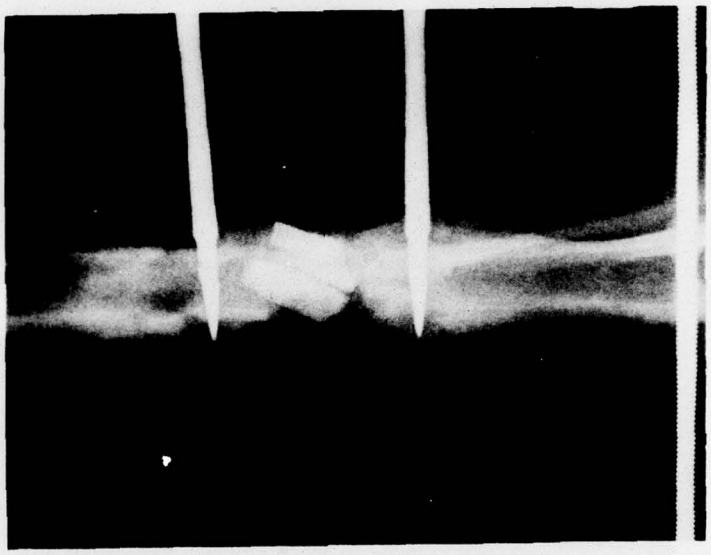


10 months

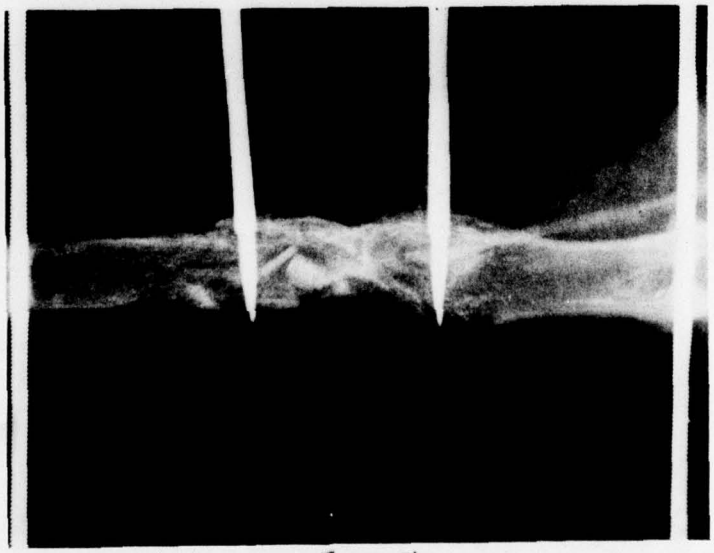
FIGURE 11: Radiographs of Nonunion Tricalcium Phosphate Ceramic, Rabbit Number 209.



2 months

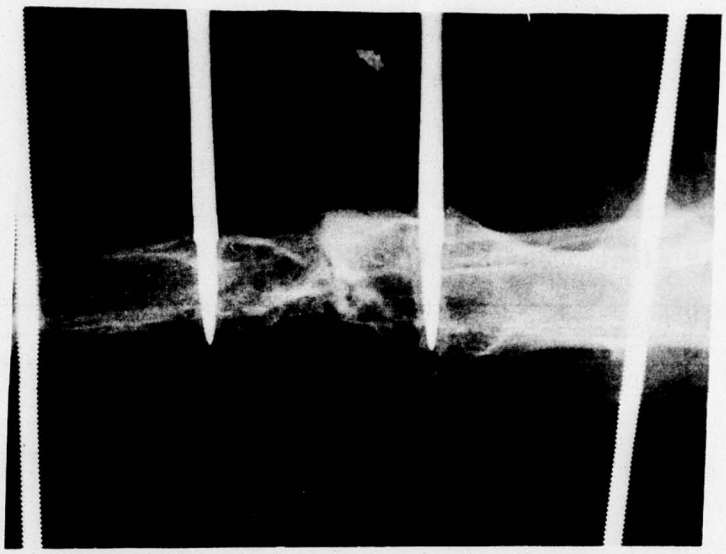


2 months

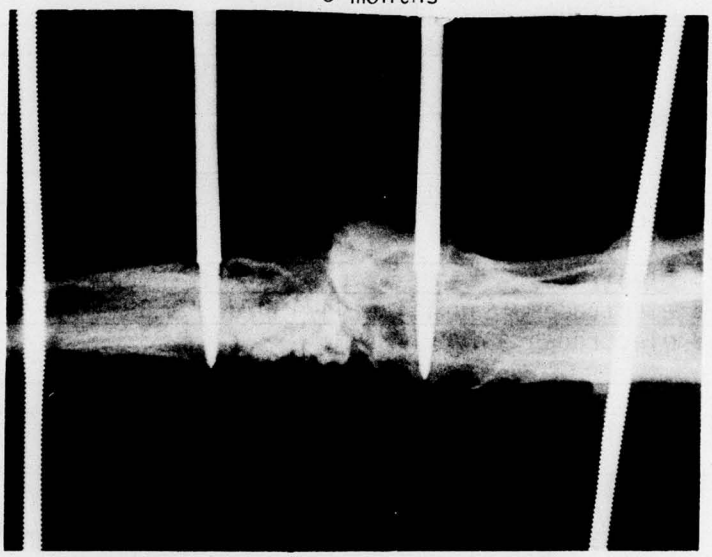


7 months

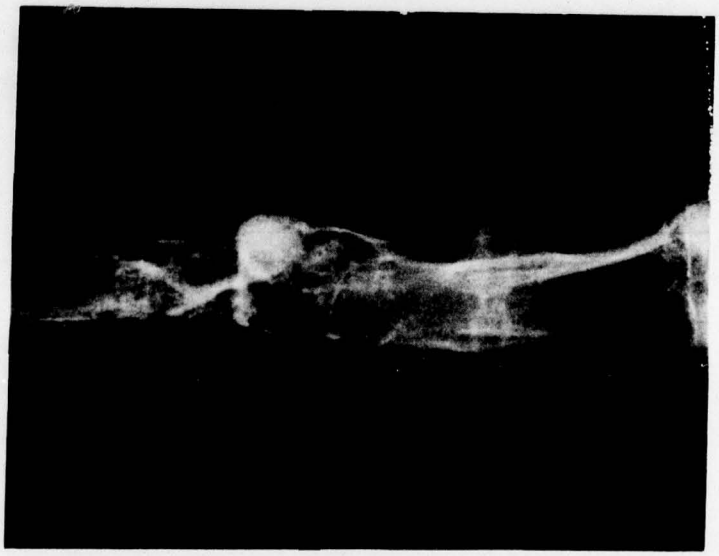
FIGURE 12: Radiographs of Tricalcium Phosphate Chip Implants for Bone Augmentation of Rabbit R219.



3 months



3 months



7 months

FIGURE 13: Radiographs of Tricalcium Phosphate "Powder" Implant for Bone Augmentation of Rabbit 217.

TABLE II. NONUNION RABBIT EXPERIMENT SERIES

RABBIT NUMBER	INITIAL SURGERY	TYPE EXPERIMENT	DISPOSITION	DURATION OF EXPER.(weeks)	COMMENTS
R205	1/16/76	NU* TiV** electrodes	Sacrifice 5/20/76	18	Infection 3/31, fracture of pin
R206	1/16/76	NU TiV electrodes	Sacrifice 3/5/76	16	Displacement
R207	1/21/76	NU TiV electrodes	Sacrifice 2/5/76	2	Displacement
R208	1/23/76	NU TiV electrodes	--	in progress	Fracture of pin 5/12/76 $Ca_3(PO_4)_2$ 7/30/76
R209	1/23/76	NU TiV electrodes	--	in progress	$Ca_3(PO_4)_2$ 7/30/76
R211	2/13/76	NU TiV electrodes	Sacrifice 12/14/76	44	Fracture electrode 6/18/76 Correction 4/16/76 $Ca_3(PO_4)_2$ 7/9/76
R212	2/13/76	NU TiV electrodes	--	in progress	$Ca_3(PO_4)_2$ 7/30/76
R213	2/27/76	NU TiV electrodes	Sacrifice 5/19/76	11	Infection 3/31/76
R214	2/27/76	NU TiV electrodes	Sacrifice 5/14/76	11	Infection
R215	3/3/76	NU TiV electrodes	--	in progress	Fracture of pin 5/12/76 $Ca_3(PO_4)_2$ 7/30/76
R216	3/5/76	NU TiV electrodes	Died 12/20/76	40	Correction 4/16/76 $Ca_3(PO_4)_2$ 7/30/76
R217	4/21/76	NU TiV electrodes	Sacrifice 11/16/76	29	$Ca_3(PO_4)_2$ 7/16/76
R218	4/21/76	NU TiV electrodes	--	in progress	Infection 6/18/76 Fracture of pin 7/7/76
R219	4/23/76	NU TiV electrodes	--	in progress	Pieces $Ca_3(PO_4)_2$ 6/25/76
R220	4/23/76	NU TiV electrodes	Sacrifice 11/19/76	30	Pieces $Ca_3(PO_4)_2$ 6/25/76
R221	4/28/76	NU TiV electrodes	Died 12/4/76	32	Infection 6/18/76 $Ca_3(PO_4)_2$ 9/9/76
R222	4/28/76	NU TiV electrodes	Sacrifice 5/14/76	2	Displacement
R223	4/30/76	NU TiV electrodes	Sacrifice 11/20/76	31	Infection 5/25/76 Pieces $Ca_3(PO_4)_2$ 6/25/76
R224	4/30/76	NU TiV electrodes	--	in progress	Infection 6/18/76 $Ca_3(PO_4)_2$ 9/9/76
R225	5/11/76	NU TiV electrodes	--	in progress	Infection 6/18/76
R226	5/12/76	NU TiV electrodes	--	in progress	
R227	5/12/76	NU TiV electrodes	--	in progress	Infection 8/12/76
R228	5/26/76	NU TiV electrodes	--	in progress	
R229	5/26/76	NU TiV electrodes	Sacrifice 8/23/76	8	Infection 8/4/76 Pieces $Ca_3(PO_4)_2$ 8/9/76
R230	5/28/76	NU TiV electrodes	--	in progress	Pieces implanted 10/22/76
R231	6/2/76	NU TiV electrodes	Died 7/2/76	4	Cause not determined
R232	6/11/76	NU TiV electrodes	--	in progress	Pieces implanted 10/22/76
R233	6/17/76	NU TiV electrodes	--	in progress	Fracture of pin 8/12/76
R234	6/18/76	NU TiV electrodes	Sacrifice 11/3/76	22	Infection 7/14/76
R235	6/18/76	NU TiV electrodes	--	in progress	Infection, drained 8/9/76 $Ca_3(PO_4)_2$ 8/9/76
R236	6/18/76	NU TiV electrodes	--	in progress	Displacement 7/14/76
R237	6/22/76	NU TiV electrodes	--	in progress	$Ca_3(PO_4)_2$ 9/9/76
R238	6/22/76	NU TiV electrodes	Died 12/5/76	24	Infection 8/12/76 Pieces implanted 11/19/76

* NU = Nonunion

** TiV = Tivanium® (Ti-6Al-4V) alloy electrodes.

TABLE III. BIOMECHANICAL STRENGTH DATA FOR 3, 6, 12 and 64 WEEKS
TRICALCIUM PHOSPHATE CERAMIC STUDY

ANIMAL	DURATION OF EXPERIMENT (wks)	WORK AT FRACTURE (lbs)	WORK TO FRACTURE	COMMENTS
R196	3	-----No Strength-----		
R197	3	-----No Strength-----		
R198	3	8	46	
R199	3	26	51	
R200E	3	8	18	
R201E	3	12	40	
R202E	3	-----No Strength-----		
R203E	3	10	29	
R204E	3	-----No Strength-----		
R189C	3	30	74	
R190C	3	40	135	
R191C	3	-----No Strength-----		
R192C	3	18.5	52	
R193C	3	-----No Strength-----		
R149	6	61	133	
R153	6	29	74	
R181	6	19	43	
R182	6	71	296	
R195	6	19.5	28	
R171E	6	25	46	
R172E	6	26	74	
R173E	6	48	187	
R174E	6	41	132	
R175E	6	8	87	
R184C	6	71	275	
R185C	6	41	446	
R186C	6	24.5	184	
R187C	6	27	77	
R188C	6	-----No Strength-----		Slight tibial displacement.
R146	12	58	141	
R147	12	26	73	
R148	12	-----No Strength-----		Infection.
R178	12	60.5	223	
R179	12	48.5	90	
R180	12	58	215	
R167E	12	3.5	21	Pins remained in.
R168E	12	-----No Strength-----		Pins remained in.
R169E	12	35	255	Pins remained in.
R170E	12	34	270	Pins remained in.
R162C	12	89	315	
R164C	12	34	179	
R176C	12	31	218	
R177C	12	59	180	
R183C	12	27	73	
R139	64	63	116	
R143	42	--	--	Died.
R144	64	74	372	
R145	50	68.5	190	Sick; euthanized.
R150E	42	51	163	Died.
R152E	64	85	364	Fracture distal to lesion.
R154E	64	47	252	
R155E	Lost	--	--	Lost.
R156E	Lost	--	--	Lost.
R118C	64	82	468	
R137C	64	97.5	498	
R158C	64	-----Not Tested------		Fractured tibia,remodeled.
R160C	64	52.5	422	

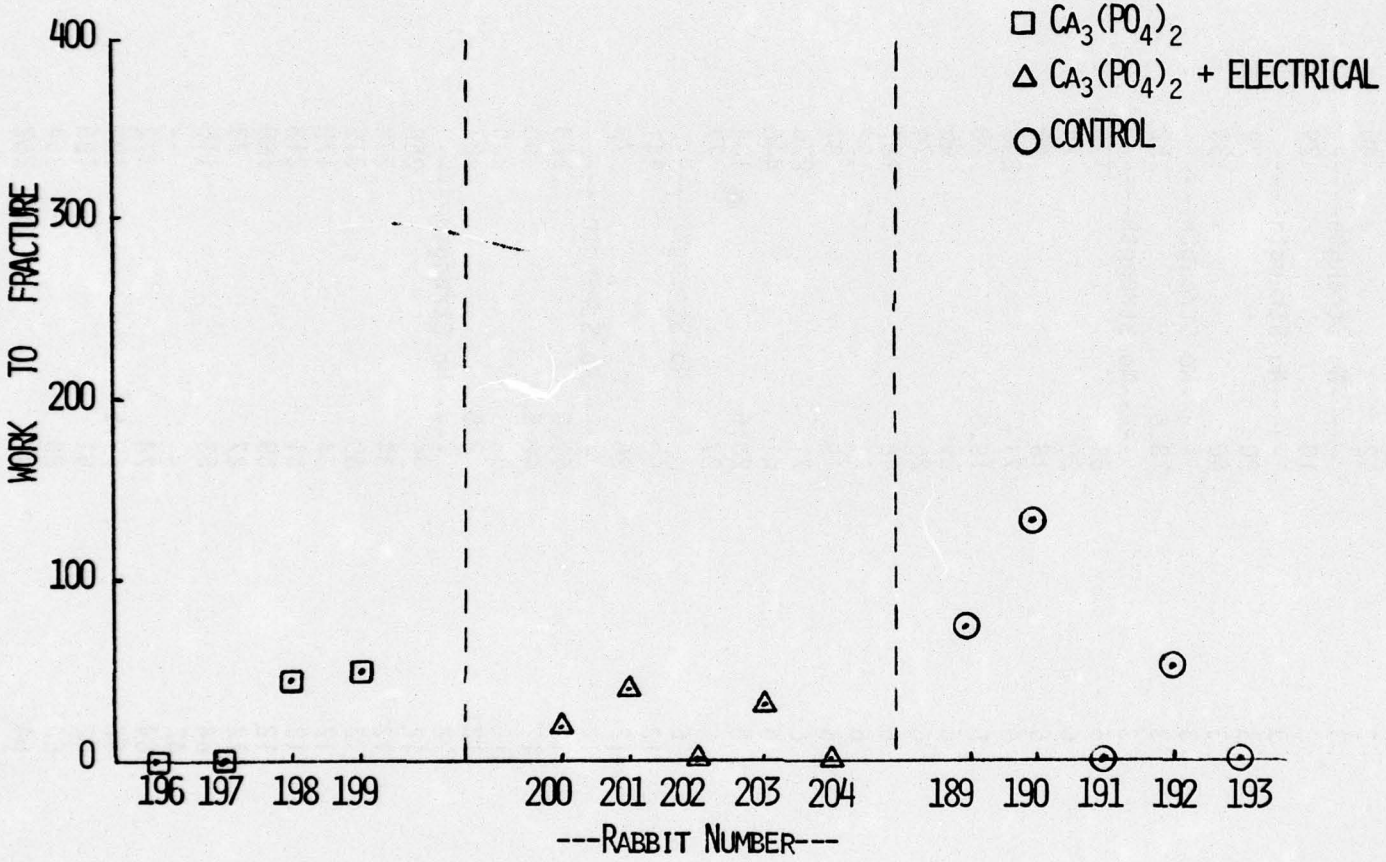


FIGURE 14. BIOMECHANICAL STRENGTH COMPARISONS AT 3 WEEKS.

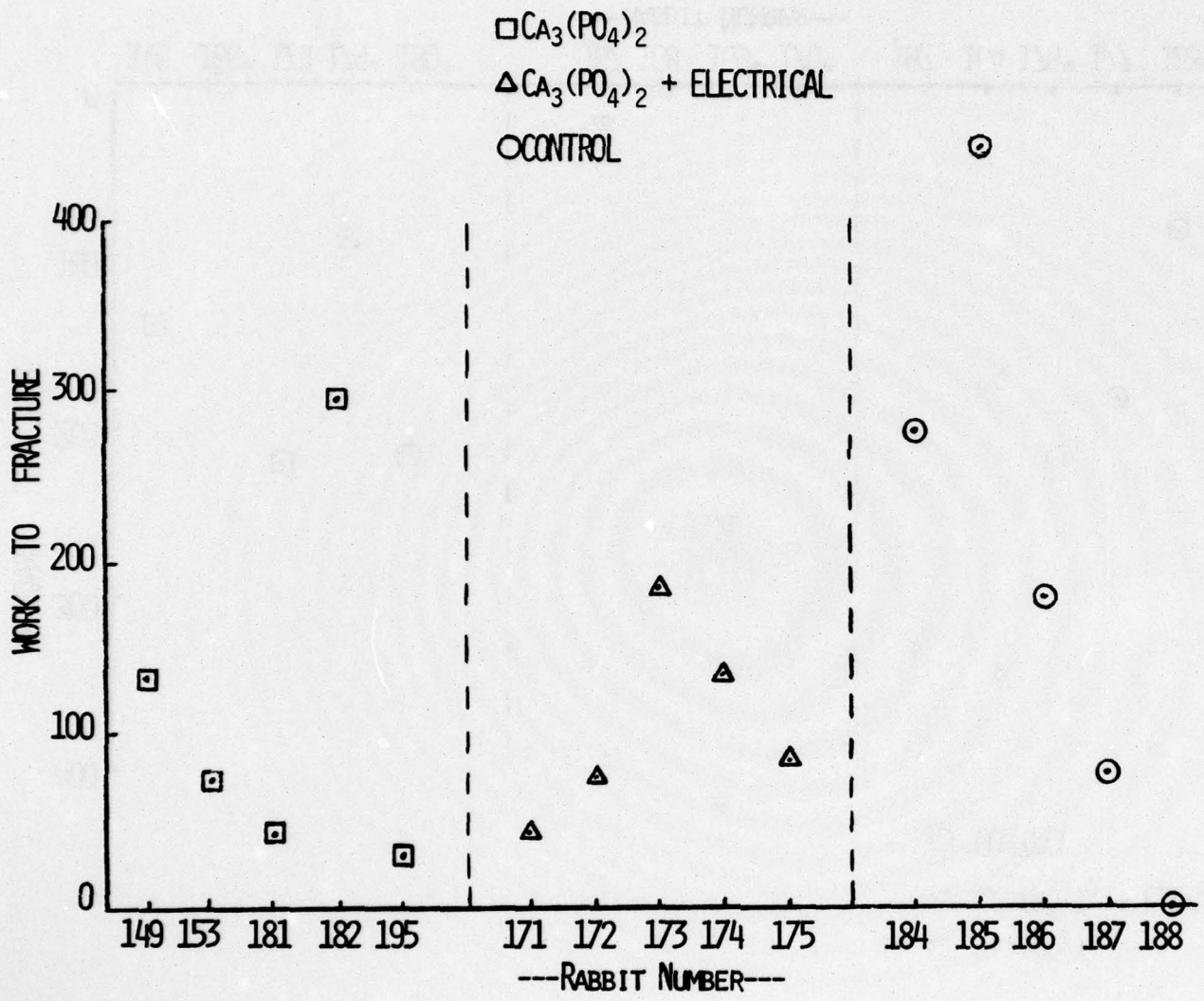


FIGURE 15. BIOMECHANICAL STRENGTH COMPARISONS AT 6 WEEKS.

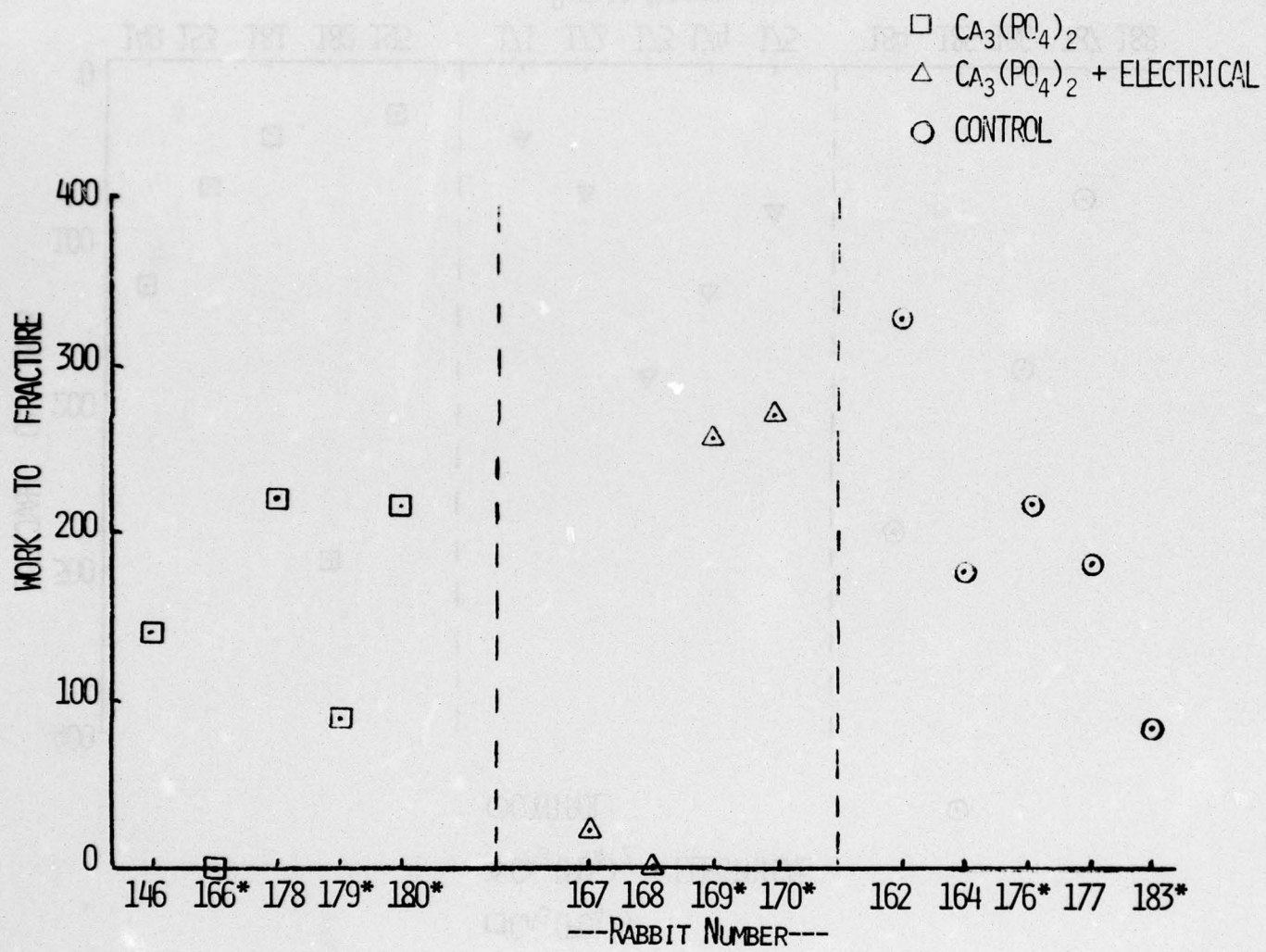


FIGURE 16. BIOMECHANICAL STRENGTH COMPARISONS AT 12 WEEKS

* STABILIZATION REMAINED

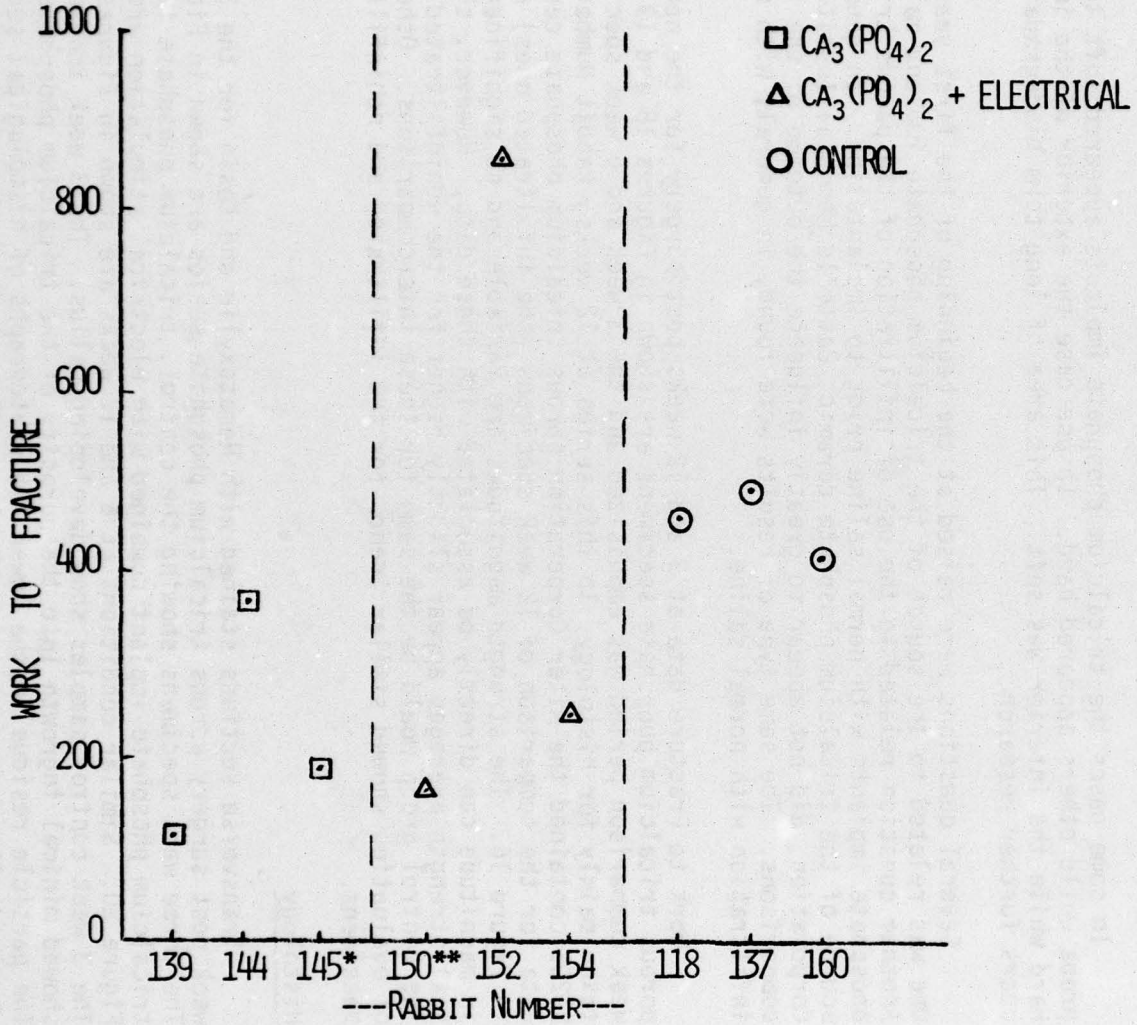


FIGURE 17. BIOMECHANICAL STRENGTH COMPARISONS AT 64 WEEKS.
*No. 145 = 50 WKS. **No. 150 = 42 WKS.

at a given time period. Note the Work to Fracture scale magnitude change for the 64 week specimens. In general, the strength increased with increasing time and the scatter in the data was considerable. This scatter can be correlated with the variable anatomy found for the rabbit tibias.

The rabbits in the 12 and 64 week groups had been using their limbs without difficulty since the 6th week post surgery. In the situations where the implant had not biodegraded, cortical bone had formed around and adjacent to the tricalcium phosphate implant. The fractures in four point bending often crossed through the implant body causing complete fracture of the implant and the bone site.

In some cases the tricalcium phosphate implants appeared soft to a sharp probe while others appeared hard. In one case the exterior of the implant was hard while the interior was soft. This area of long term biodegradation potential bears further research.

Several questions were raised at the beginning of the first year program. One was related to the source of the tricalcium phosphate implant material. Another question related to the use of infiltration of the porous tricalcium phosphate implants with normal saline prior to implantation. In general, the source of the tricalcium phosphate ceramic, Battelle Memorial Institute or Miter Corporation, did not appear to greatly influence the outcome of the implantation comparisons. The same type of results were found, in general, with respect to infiltration with normal saline.

Work to Fracture data at 3 and 12 weeks post surgery for the noninfiltrated porous tricalcium phosphate specimens are shown in Figures 18 and 19. The 12 week comparison period was emphasized and the 3 week and 6 week specimens were taken mainly for histology. In this series at 12 weeks, Rabbit Number R119 and R120E contained the Miter Corporation porous tricalcium phosphate ceramic. The data for the comparison of 12 week specimens (the infiltrated ones) are shown in Figure 16. The strength magnitudes are variable and no significant difference in magnitude can directly be associated with these data. However, the biomechanical strength averages appear slightly higher for the noninfiltrated specimens. The control group would be the same for these intercomparisons. Other methods of evaluation showed similar trends for the infiltrated and noninfiltrated specimens.

Histology

Transverse sections stained with Hematoxylin and Eosin for the 3, 6, and 12 week post surgery porous tricalcium phosphate series are shown in Figures 20-22. The three week specimens showing the control, tricalcium phosphate implant, and tricalcium phosphate implant combined with electrical stimulation are shown in Figure 20. Similar conditions at 6 and 12 weeks are shown in Figures 21 and 22. The 3 week control samples show developing callus. The 3 week implant conditions showed minimal ingrowth into the porosity of the tricalcium phosphate ceramic. The particle residue in the lower two photographs of histological sections remained after processing and should be disregarded. The empty regions represent the locations where the tricalcium phosphate sample was removed during specimen preparation.

The 6 week control sample shows developed callus as would be expected. The implant samples show limited tissue ingrowth into the tricalcium phosphate

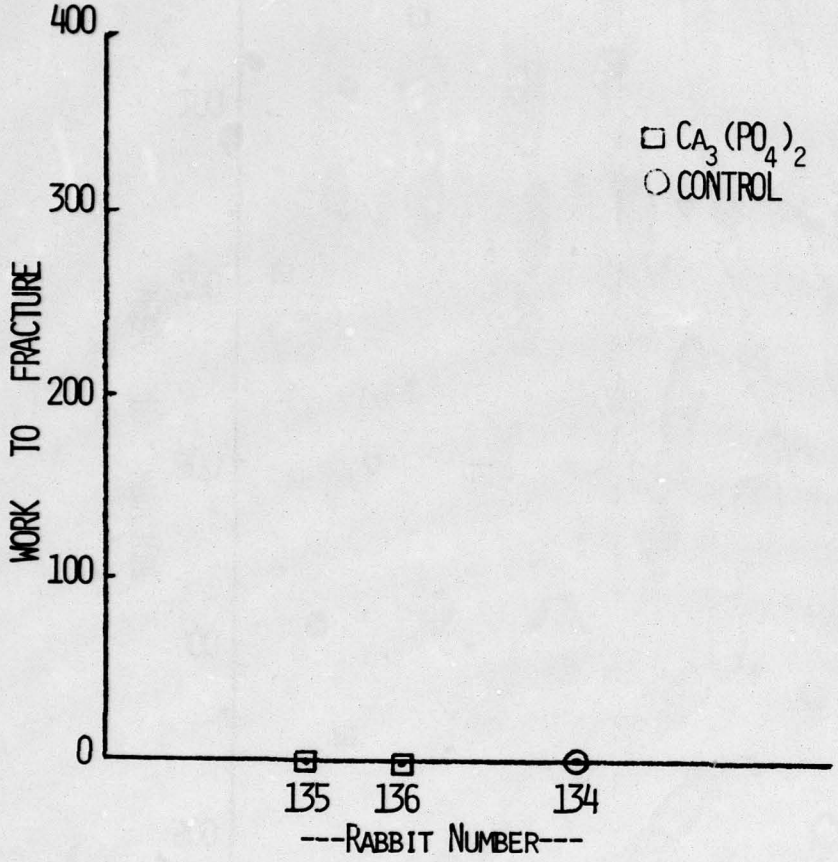


FIGURE 18. BIOMECHANICAL STRENGTH COMPARISONS FOR NON-SALINE INFILTRATED TRICALCIUM PHOSPHATE CERAMIC SPECIMENS AT 3 WEEKS POST SURGERY

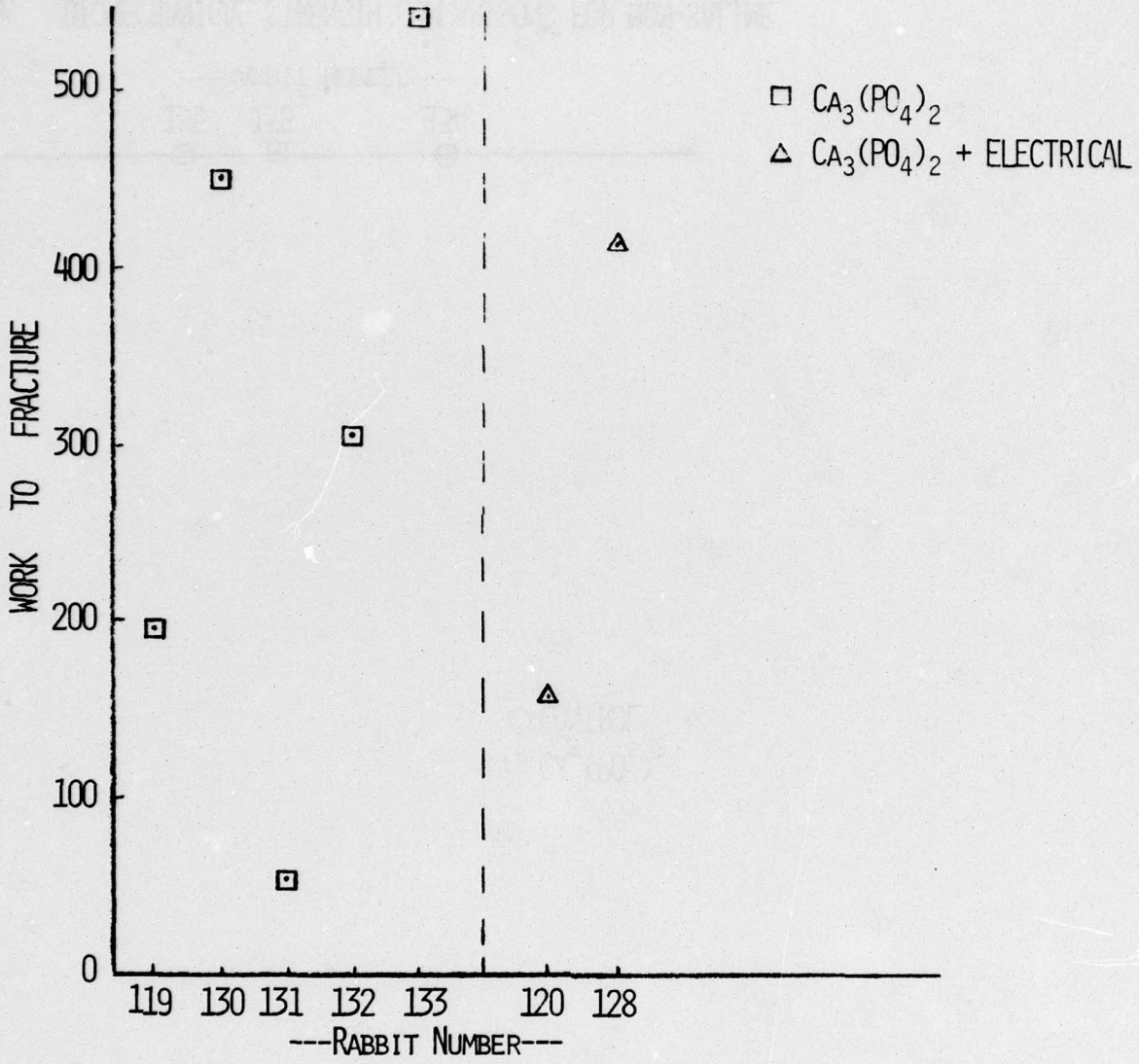
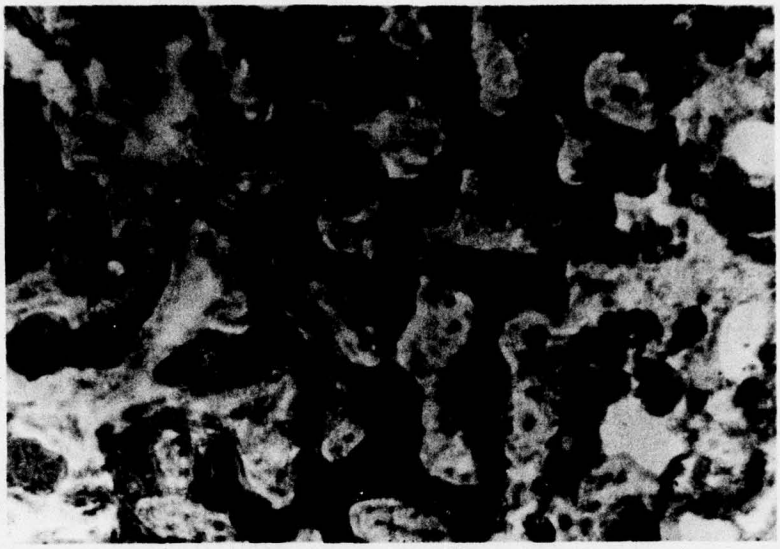
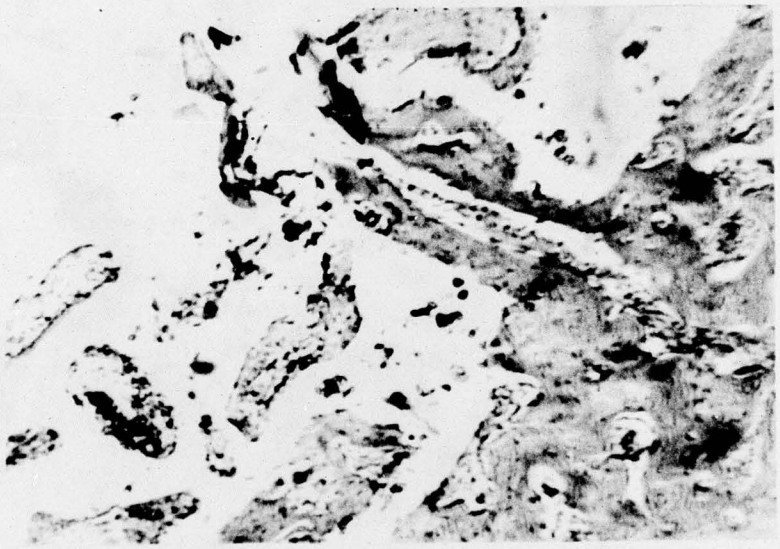


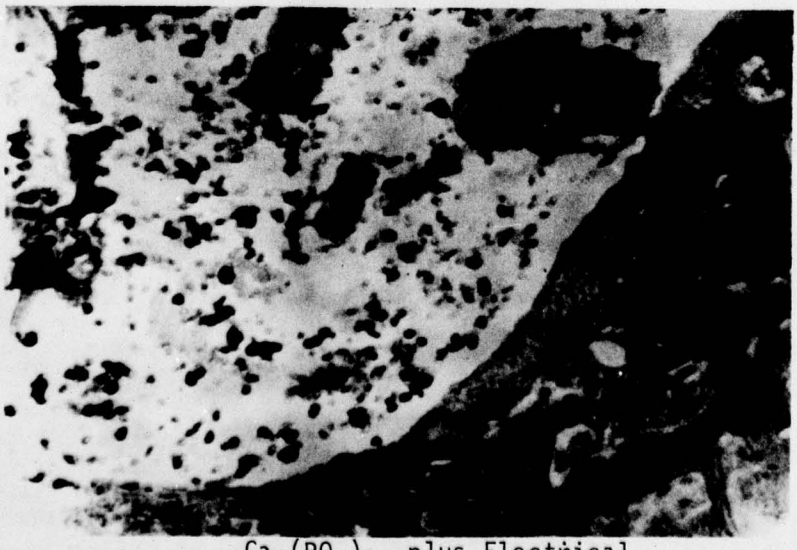
FIGURE 19. BIOMECHANICAL STRENGTH COMPARISONS FOR NON-SALINE INFILTRATED TRICALCIUM PHOSPHATE CERAMIC SPECIMENS AT 12 WEEKS



Control

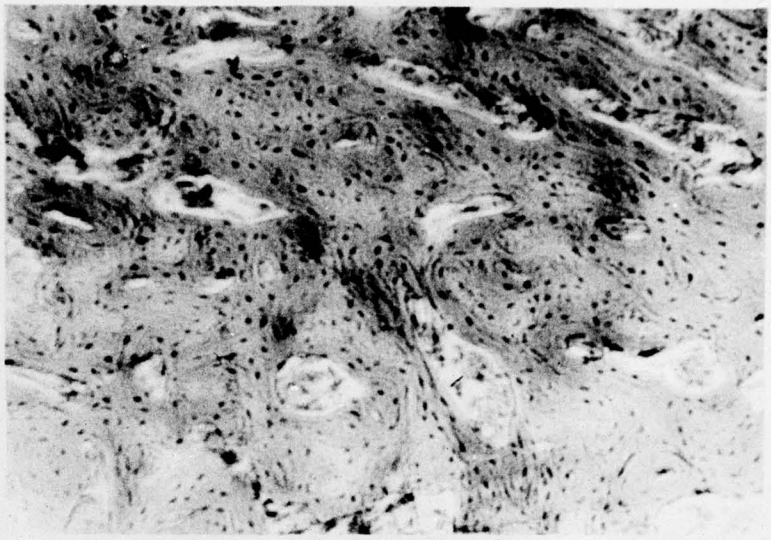


$Ca_3(PO_4)_2$



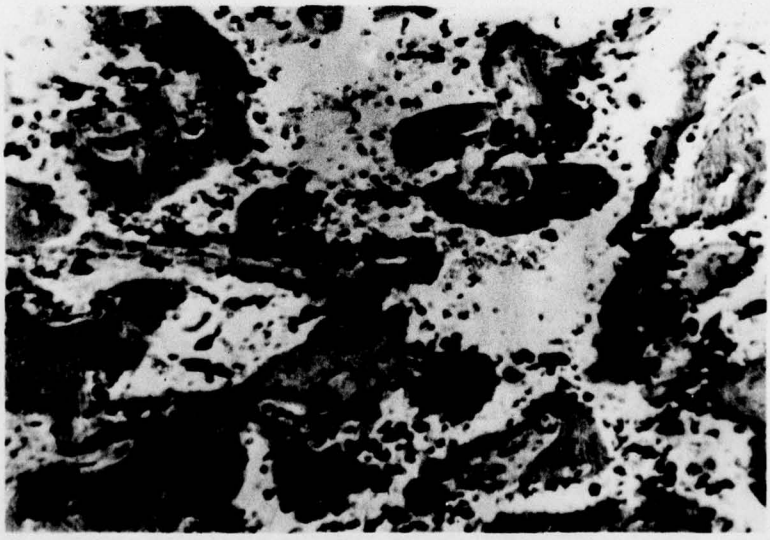
$Ca_3(PO_4)_2$ plus Electrical

FIGURE 20. Hematoxylin and Eosin Stained Transverse Histological Specimens for the 3 Week Comparisons.



Control

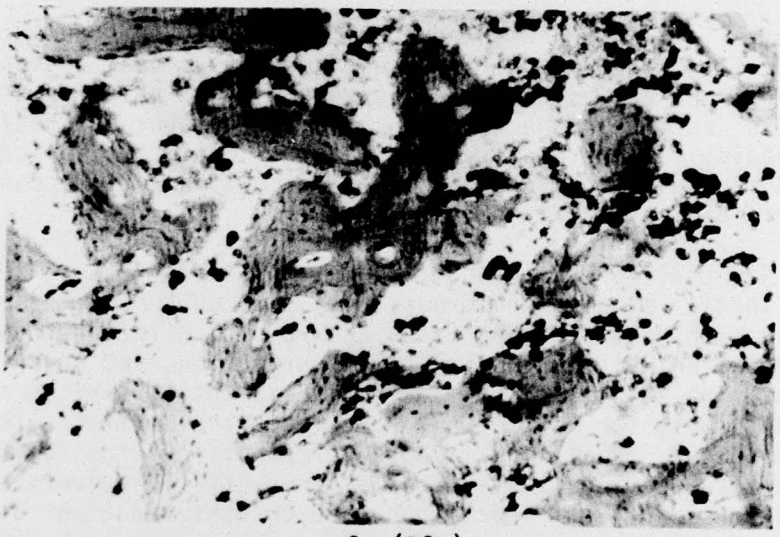
$Ca_3(PO_4)_2$



$Ca_3(PO_4)_2$ plus Electrical

FIGURE 21. Hematoxylin and Eosin Stained Transverse Histological Specimens for the 6 Week Comparisons.

Control



$Ca_3(PO_4)_2$

$Ca_3(PO_4)_2$ plus Electrical

FIGURE 22. Hematoxylin and Eosin Stained Transverse Histological Specimens for the 12 Week Comparisons.

porosity with bone structure occupying a significant portion of the perimeter of the porous implants. The amount of ingrowth under conditions of electrical stimulation did not appear greater in comparison to the nonelectrically stimulated condition.

The twelve week specimens, Figure 22, showed developed callus for the control and considerable ingrowth in the tricalcium phosphate implants. Again, the electrically stimulated and nonstimulated conditions were similar.

In all samples, the control and experimental tissues showed similar conditions. In general, the tissues showed biocompatible conditions with the tricalcium phosphate implant materials.

CONCLUSIONS

Conclusions from the present investigations on porous tricalcium phosphate ceramic are as follows:

1. Biomaterial analyses showed that comparisons of structure by x-ray diffraction produced differences in relative peak intensities at selected 2θ angles but relatively consistent patterns sample to sample; an interconnected porosity with an average cross section exceeding 100 micrometers; the material could be fabricated to produce implant designs; and no difficulties were encountered in sterilizing or handling the material.

2. The New Zealand White rabbit animal model provides an adequate model for initial studies on porous tricalcium phosphate ceramic for evaluation of tissue ingrowth, the role of direct current electrical stimulation, biodegradation, tissue reaction, and nonunion replacement. The ability to remove the stabilization devices at 6 weeks post surgery for most of the rabbits is the earliest time we have experienced. Most "inert" porous implants require 12-16 weeks. Immediate post operative care was uneventful; however, some transcutaneous pin tract infections were encountered after 3-6 weeks. These problems were severe for some of the long term nonunion animals. After removal of transcutaneous devices, the remainder of the animal care was routine.

3. This porous tricalcium phosphate ceramic in this animal model can serve as a scaffold with bone proliferation through the large interconnecting pores. Transverse sections showed relatively complete ingrowth of bone at 12 weeks.

4. Radiographs and gross observation at necropsy showed considerable variability in the rate of tricalcium phosphate implant biodegradation. Some animals retained most of the implant after 64 weeks of implantation while others showed almost complete biodegradation. The implants showed hard and/or soft conditions by sharp probe examination. In general, the radiographic appearance of the rabbit tibias showed a steady progression toward normal anatomy after 6-12 weeks.

5. The direct current electrical stimulation resulted in more periosteal callus, and did not appear to greatly influence the tissue ingrowth and biodegradation rates for the porous tricalcium phosphate ceramic implants.

6. Biomechanical strength comparisons from four point bending and determinations of the Work to Fracture for implant and control conditions, at 3, 6, 12, and 64 weeks, showed similar ranges for the strength magnitudes at each time period. The average magnitudes of the Work to Fracture data increased with increasing time post surgery but showed a wide range within each group.

7. Gross observation of implant sites at necropsy showed minimal tissue reaction while histological evaluations showed similar tissue characteristics for experimental and control conditions.

8. Comparisons of porous tricalcium phosphate implants and implants plus direct current electrical stimulation at nonunions showed quite variable conditions and a very low probability for reestablishment of bony union after porous tricalcium phosphate ceramic implantation.

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